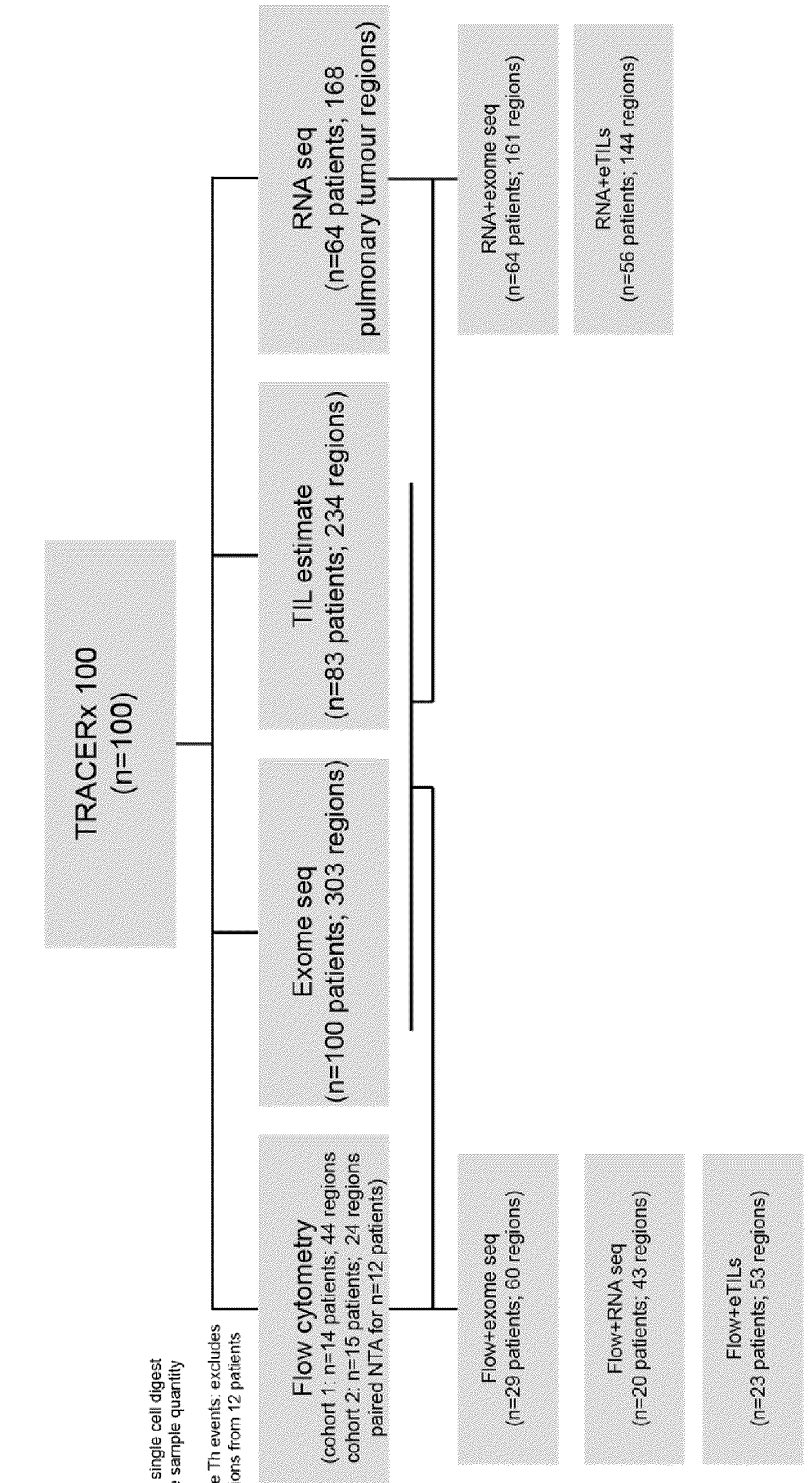

A
TRACERx 100
(n=100)
Available single cell digest
Adequate sample quantity
>1000 live Th events: excludes
n=17 regions from 12 patients
Flow cytometry
(cohort 1: n=14 patients; 44 regions
cohort 2: n=15 patients; 24 regions
paired NTA for n=12 patients)
Exome seq
(n=100 patients; 303 regions)
TIL estimate
(n=83 patients; 234 regions)
RNA seq
(n=64 patients; 168
pulmonary tumour regions)
Flow+exome seq
(n=29 patients; 60 regions)
Flow+RNA seq
(n=20 patients; 43 regions)
Flow+eTILs
(n=23 patients; 53 regions)
RNA+exome seq
(n=64 patients; 161 regions)
RNA+eTILs
(n=56 patients; 144 regions)

Figure 1

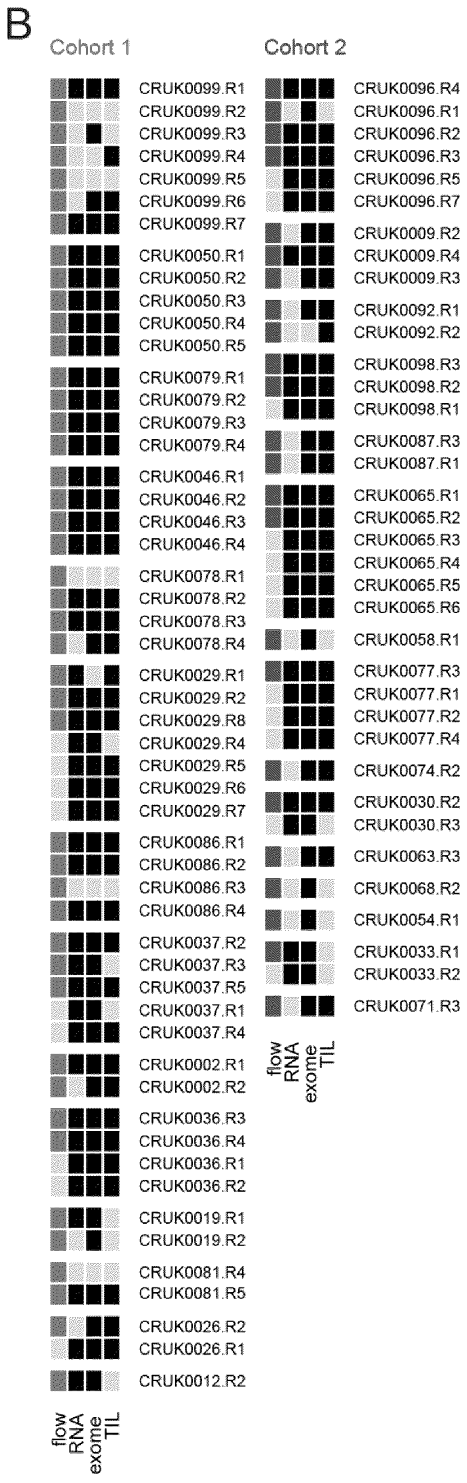


Figure 1 (continued)

C

ID	Age	Gender	Histology	Stage	Tumour regions	Paired normal	Cohort
CRUK0099	77	Male	Adenosquamous carcinoma	3a	7	+	1
CRUK0050	55	Male	Adenocarcinoma	3a	5		1
CRUK0079	63	Female	Squamous cell carcinoma	2a	4	+	1
CRUK0046	61	Female	Adenocarcinoma	2a	4	+	1
CRUK0078	58	Male	Squamous cell carcinoma	1b	3	+	1
CRUK0029	56	Male	Adenocarcinoma	3a	3		1
CRUK0086	79	Male	Squamous cell carcinoma	1b	3	+	1
CRUK0037	83	Male	Adenocarcinoma	2b	3	+	1
CRUK0002	81	Male	Adenocarcinoma	1b	2	+	1
CRUK0036	64	Female	Adenocarcinoma	1b	2	+	1
CRUK0019	54	Female	Adenocarcinoma	1b	2	+	1
CRUK0081	72	Male	Squamous cell carcinoma	2a	1	+	1
CRUK0026	67	Female	Adenocarcinoma	1a	1	+	1
CRUK0012	65	Male	Adenocarcinoma	1a	1	+	1
CRUK0096	74	Female	Adenosquamous carcinoma	2b	4		2
CRUK0009	55	Male	Adenocarcinoma	2b	3		2
CRUK0092	76	Male	Squamous cell carcinoma	2a	2		2
CRUK0098	55	Male	Carcinosarcoma	1b	2		2
CRUK0087	81	Female	Squamous cell carcinoma	3a	2		2
CRUK0065	76	Male	Squamous cell carcinoma	2b	2		2
CRUK0058	74	Male	Adenocarcinoma	2b	1		2
CRUK0077	72	Male	Squamous cell carcinoma	1b	1		2
CRUK0074	80	Male	Squamous cell carcinoma	2b	1		2
CRUK0030	75	Male	Adenocarcinoma	3a	1		2
CRUK0063	65	Male	Squamous cell carcinoma	2a	1		2
CRUK0068	68	Female	Squamous cell carcinoma	1b	1		2
CRUK0054	76	Female	Adenocarcinoma	1a	1		2
CRUK0033	68	Male	Adenocarcinoma	1a	1		2
CRUK0071	66	Male	Squamous cell carcinoma	2a	1		2

Figure 1 (continued)

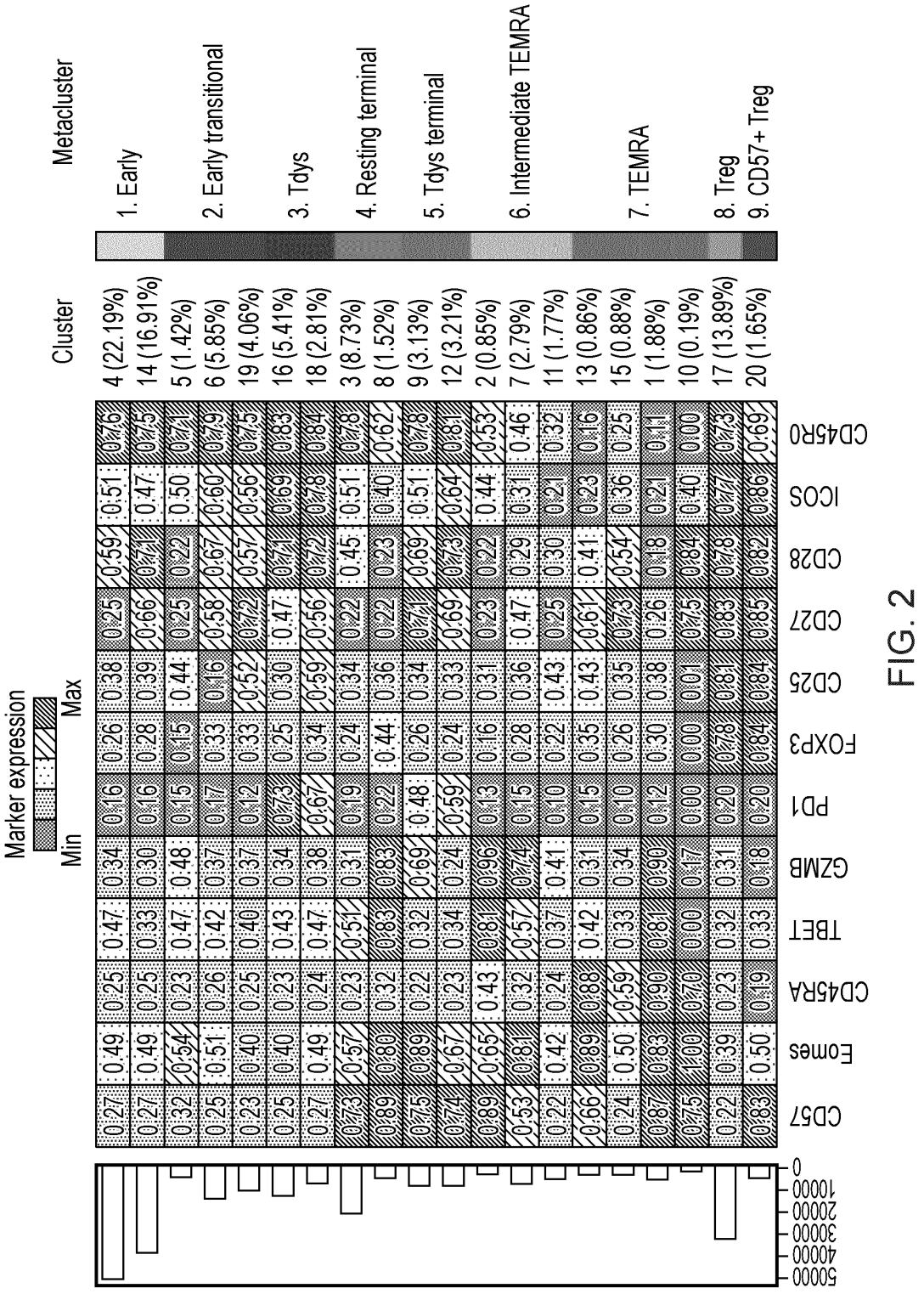

Marker expression
Min
Max
Cluster
Metacluster
4 (22.19%)
14 (16.91%)
5 (1.42%)
6 (5.85%)
19 (4.06%)
16 (5.41%)
18 (2.81%)
3 (8.73%)
8 (1.52%)
9 (3.13%)
12 (3.21%)
2 (0.85%)
7 (2.79%)
11 (1.77%)
13 (0.86%)
15 (0.88%)
1 (1.88%)
10 (0.19%)
17 (13.89%)
20 (1.65%)
1. Early
2. Early transitional
3. Tdys
4. Resting terminal
5. Tdys terminal
6. Intermediate TEMRA
7. TEMRA
8. Treg
9. CD57+ Treg
CD57
Eomes
CD45RA
TBET
GZMB
PD1
FOXP3
CD25
CD27
CD28
ICOS
CD45RO
0
10000
20000
30000
40000
50000

FIG. 2

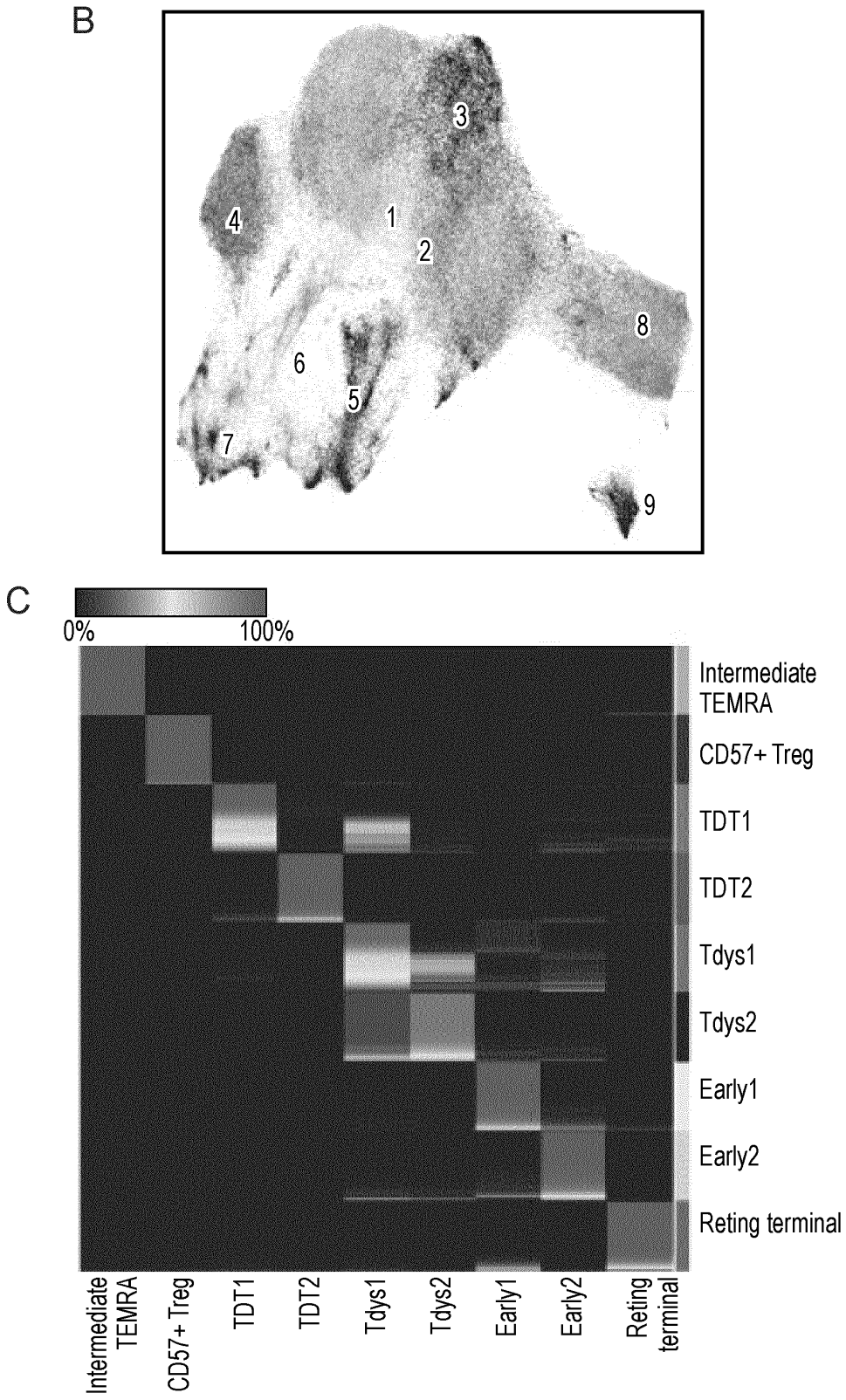


FIG. 2 (Continued)

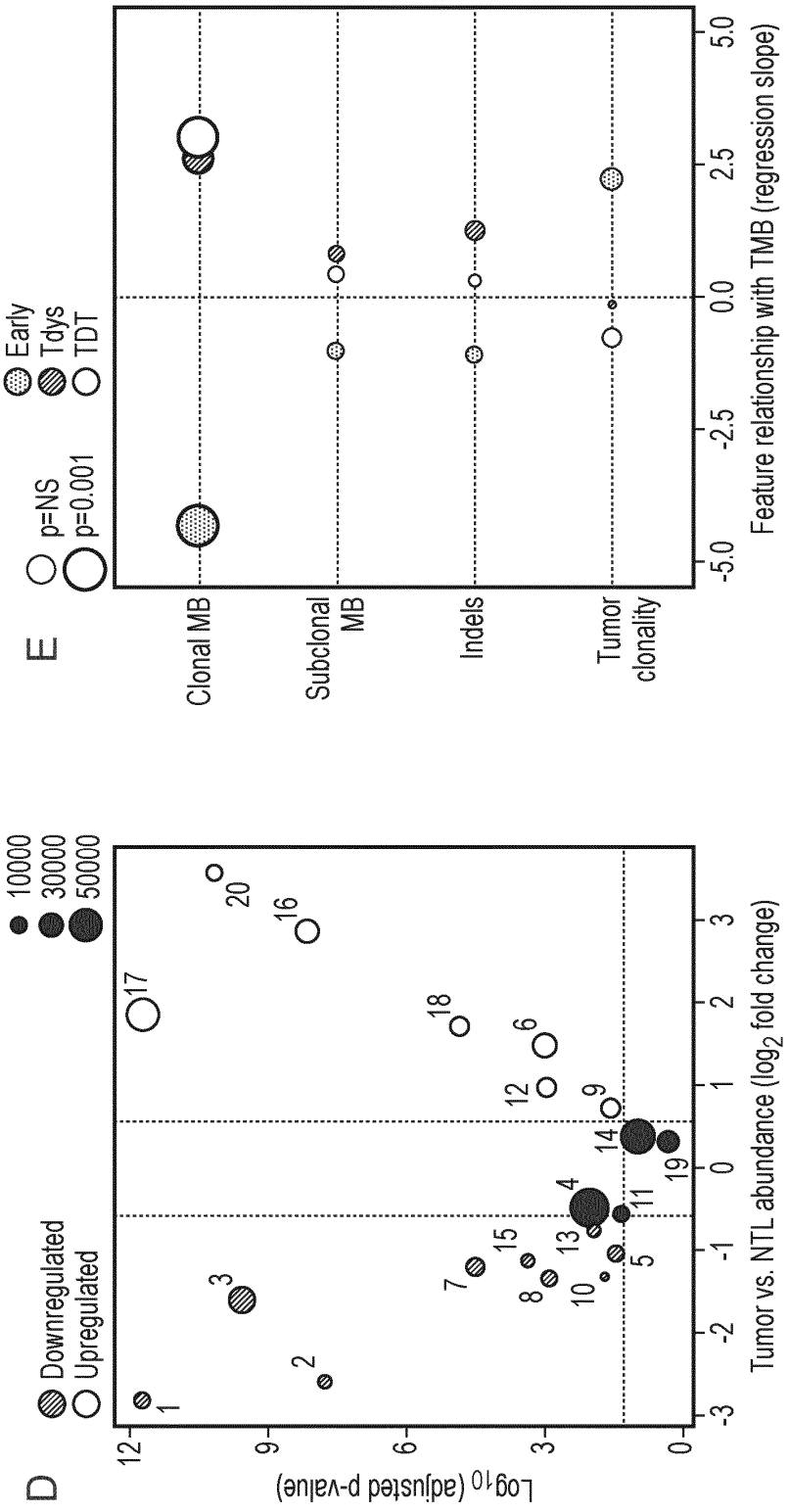
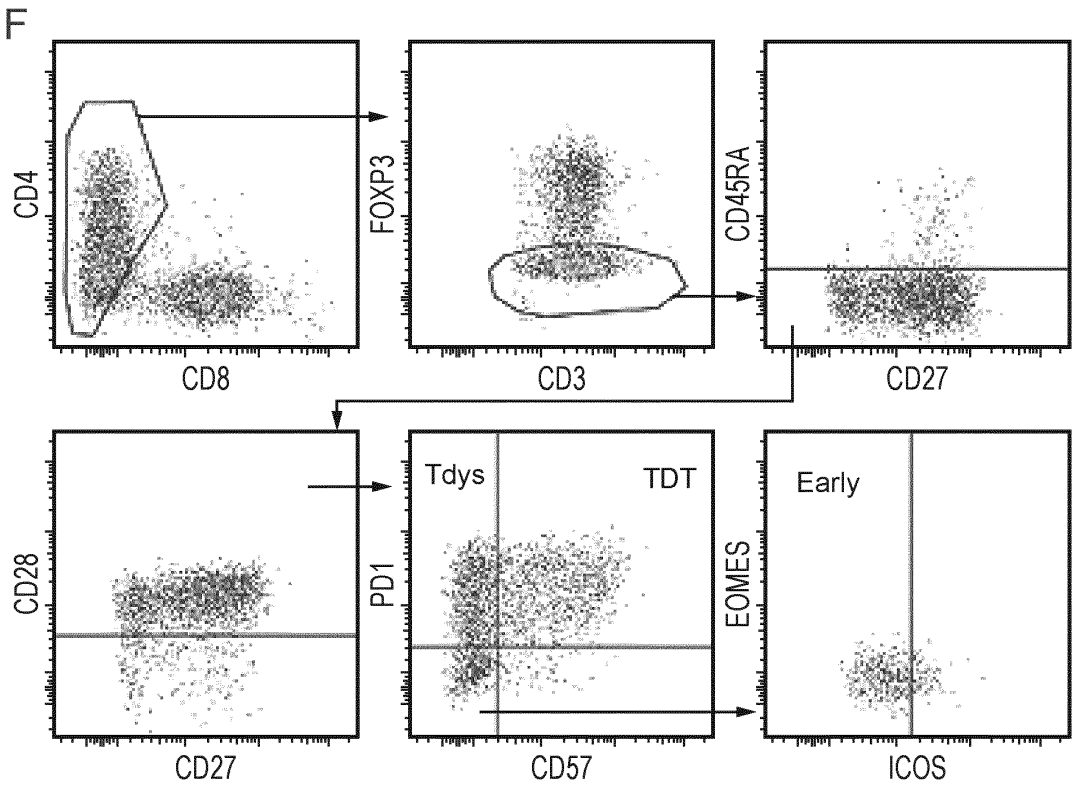


FIG. 2 (Continued)



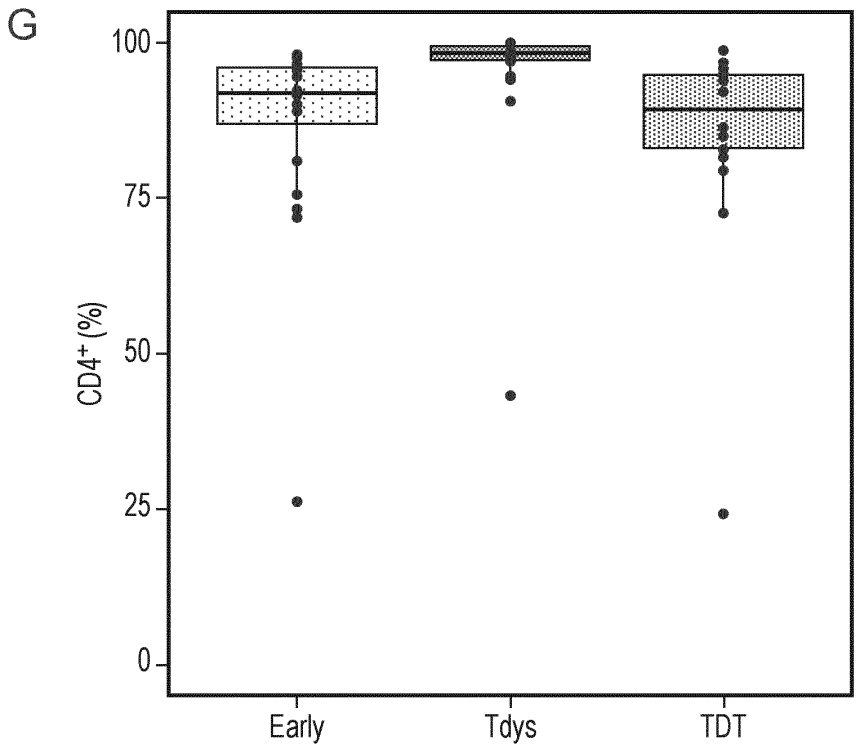


FIG. 2 (Continued)

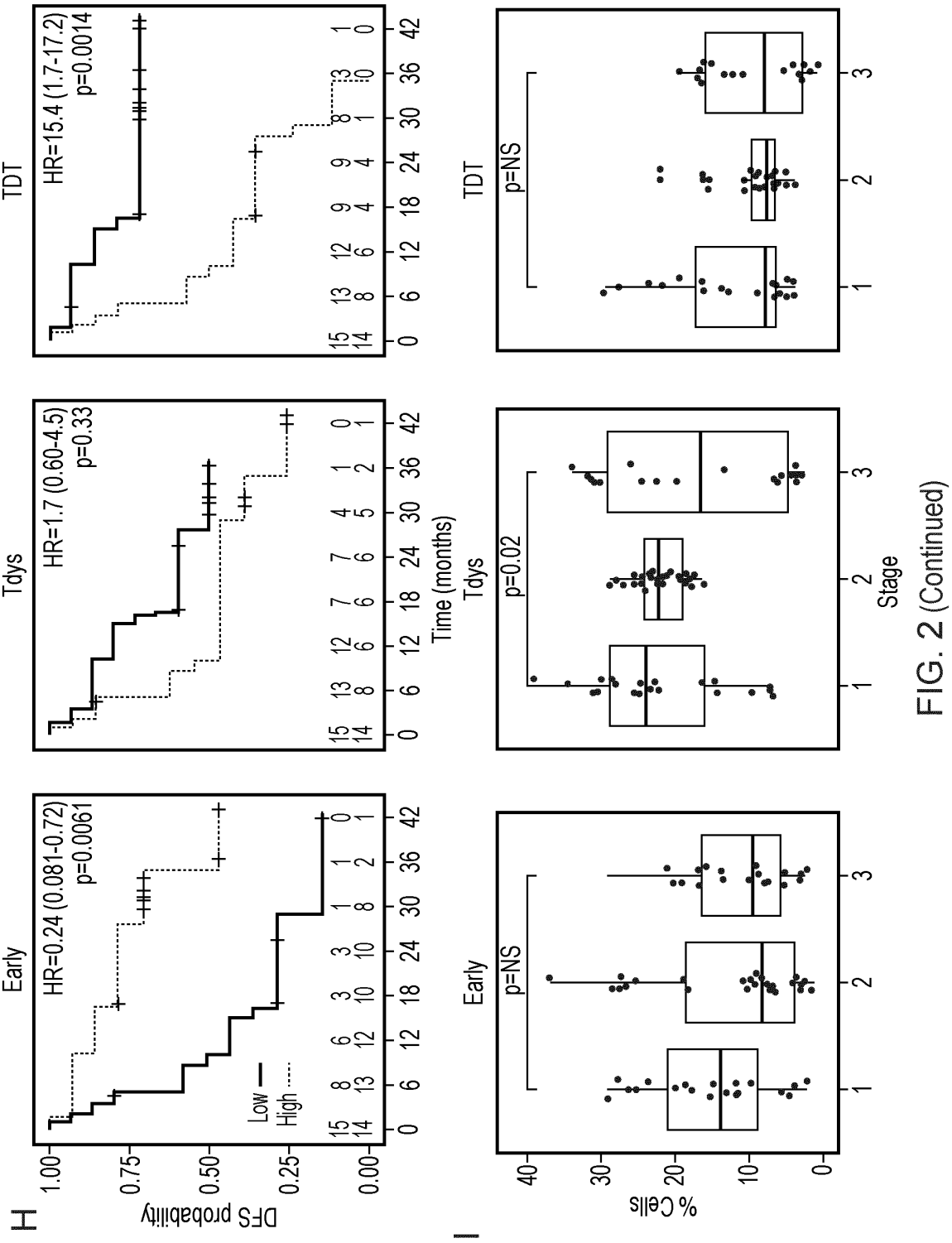


FIG. 2 (Continued)

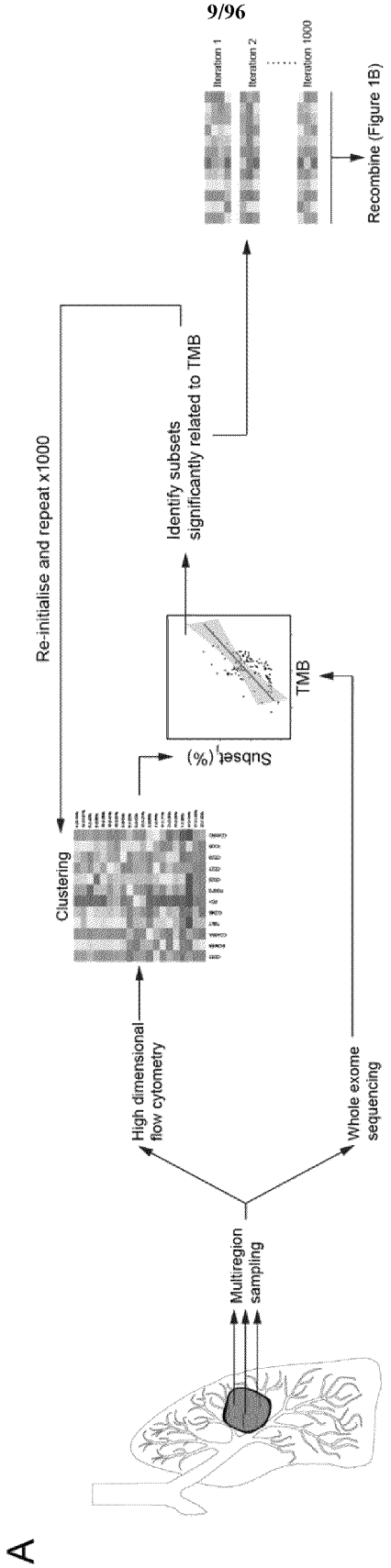

A
Multiregion sampling
High dimensional flow cytometry
Whole exome sequencing
Clustering
Subset (%)
TMB
Identify subsets significantly related to TMB
Re-initialise and repeat x1000
Iteration 1
Iteration 2
Iteration 1000
Recombine (Figure 1B)

Figure 3

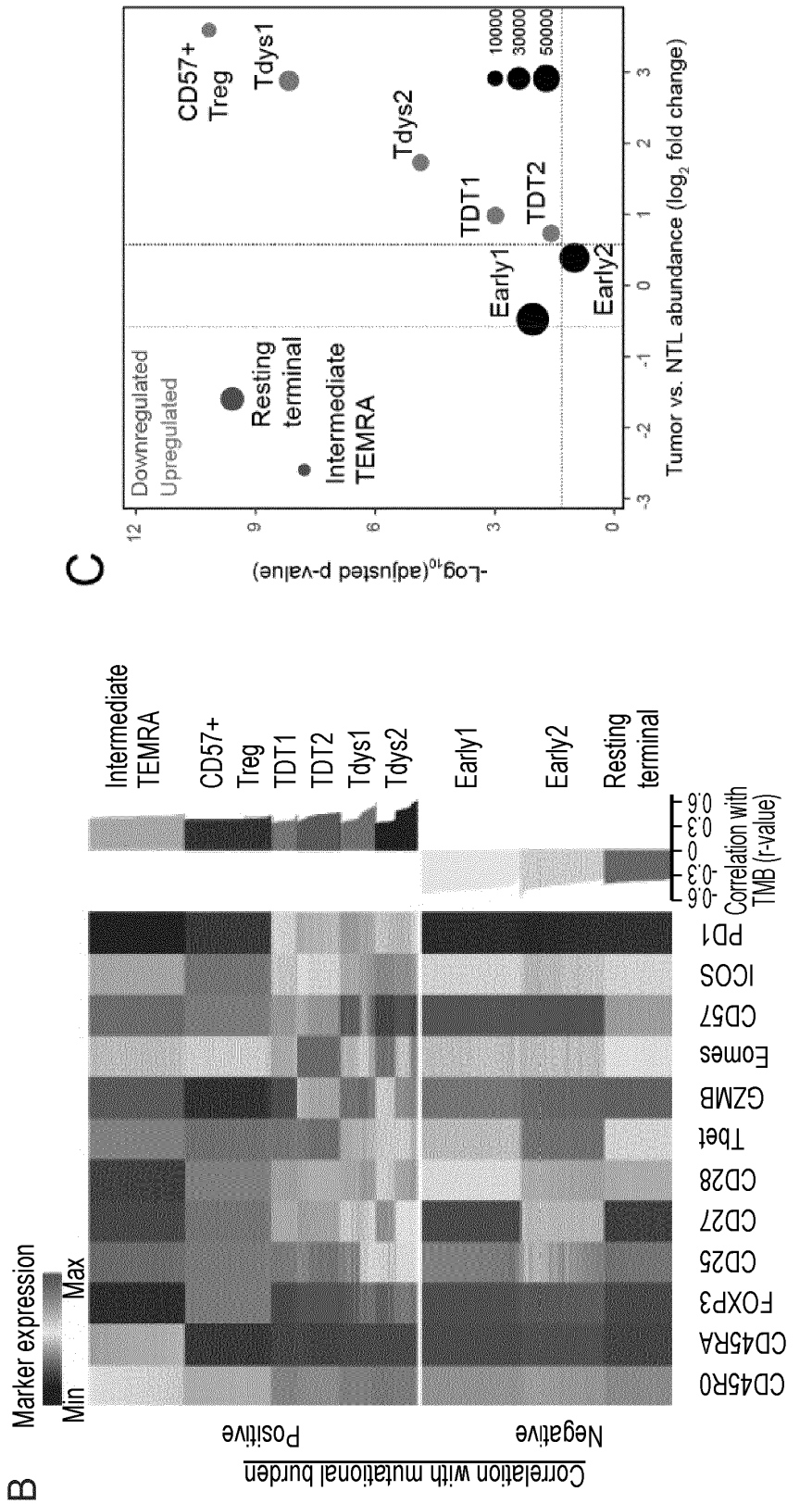


FIG. 3 (Continued)

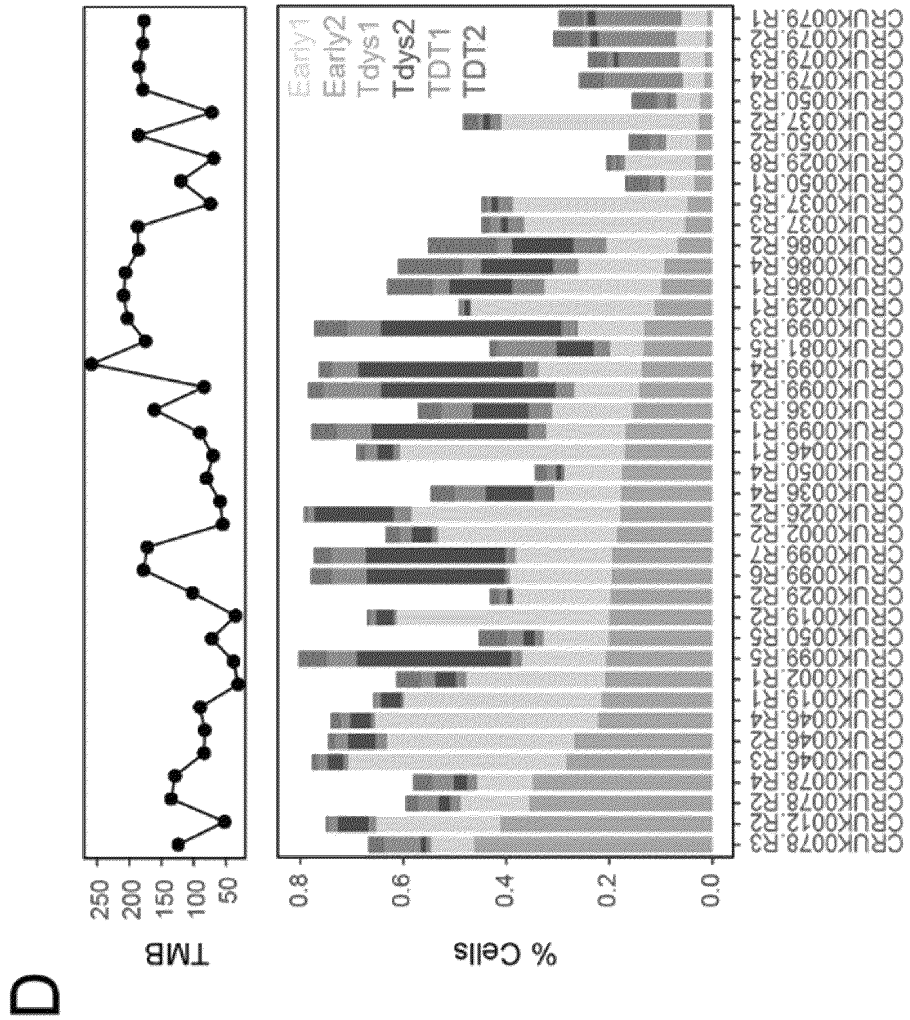


Figure 3 (continued)

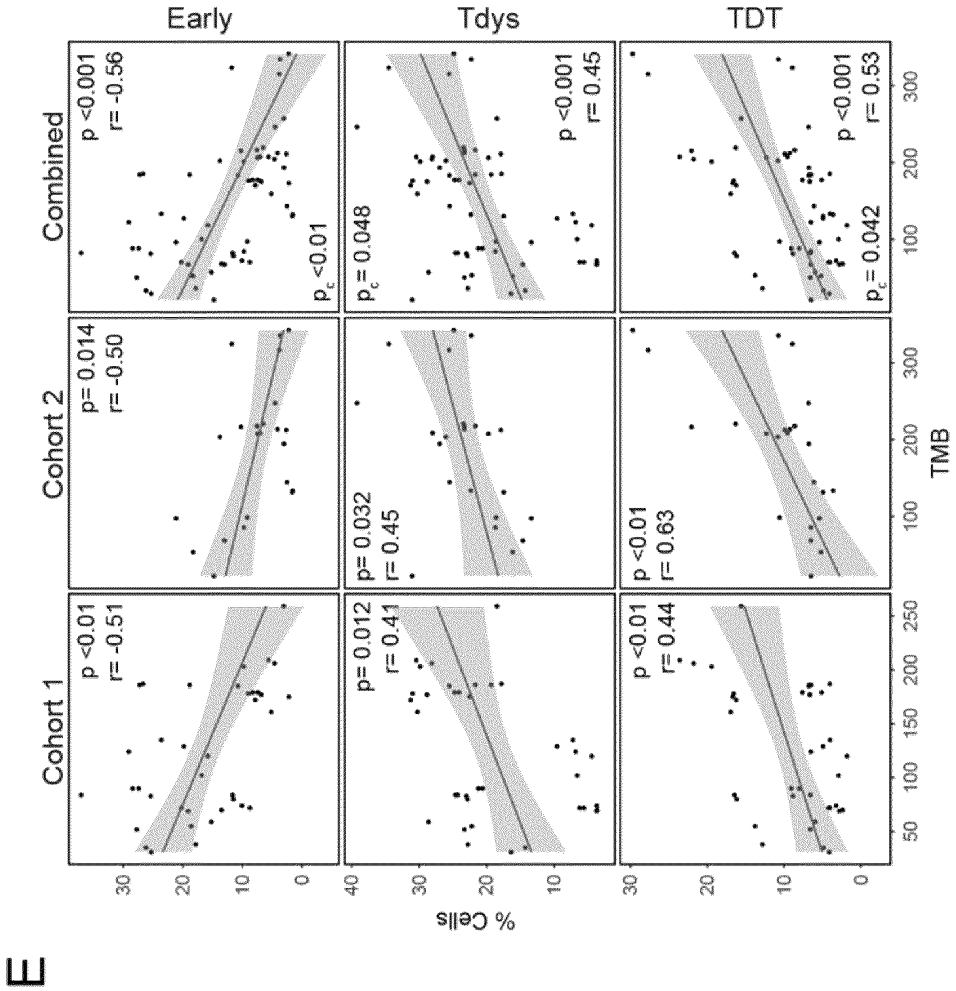


Figure 3 (continued)

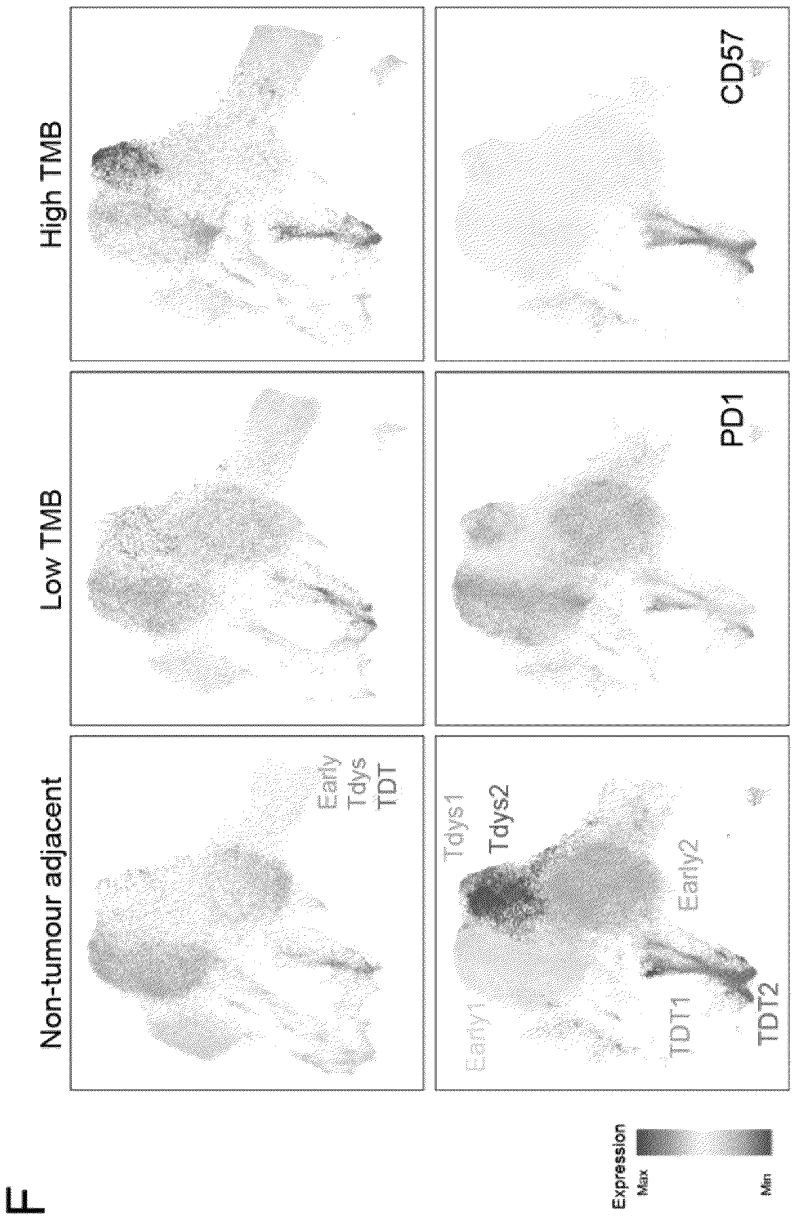


Figure 3 (continued)

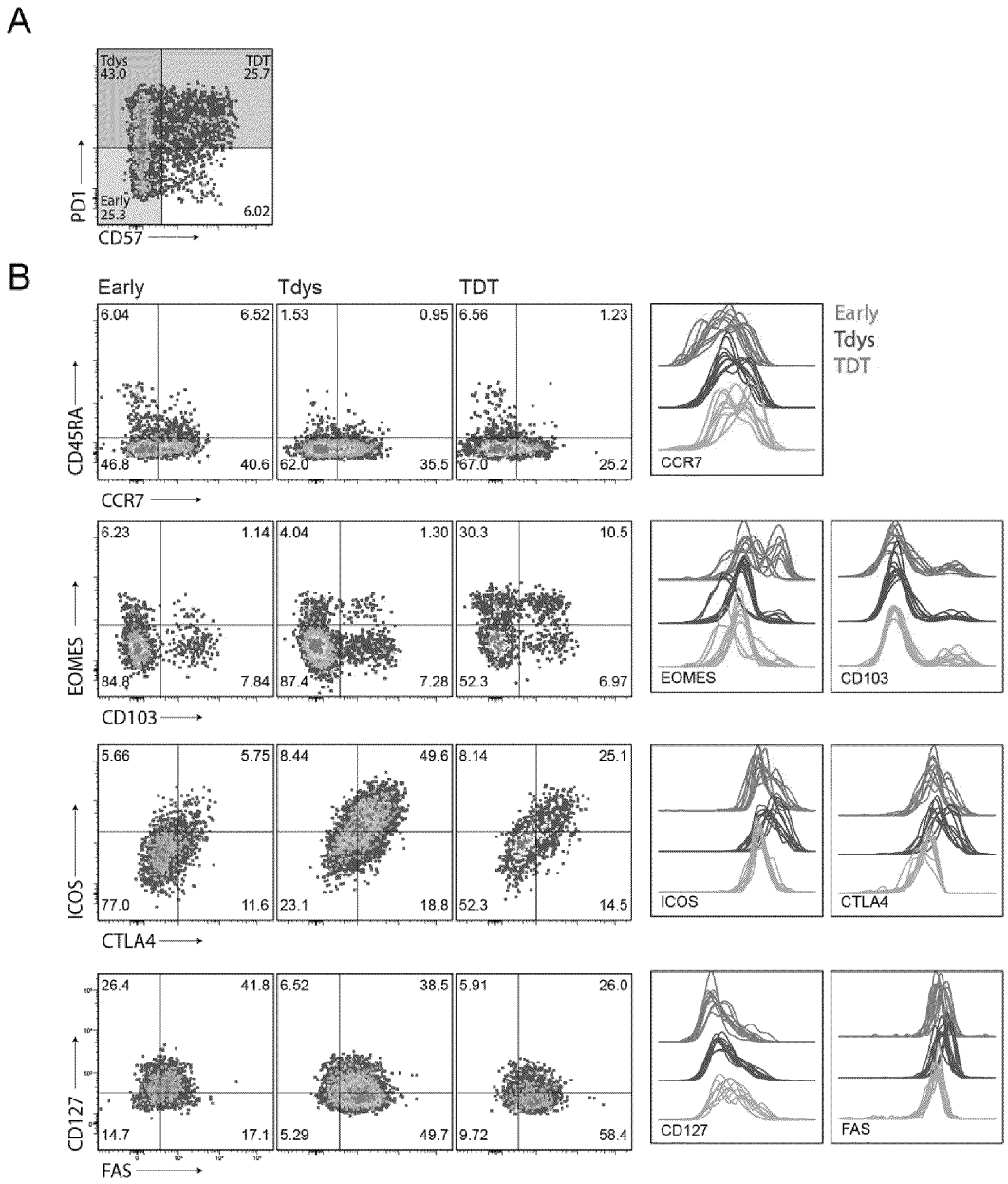

A
Tdys
43.0
TDT
25.7
Early
25.3
6.02
PD1
CD57
B
Early
Tdys
TDT
6.04
6.52
46.8
40.6
1.53
0.95
62.0
35.5
6.56
1.23
67.0
25.2
CD45RA
CCR7
Early
Tdys
TDT
CCR7
6.23
1.14
84.8
7.84
4.04
1.30
87.4
7.28
30.3
10.5
52.3
6.97
EOMES
CD103
EOMES
CD103
5.66
5.75
77.0
11.6
8.44
49.6
23.1
18.8
8.14
25.1
52.3
14.5
ICOS
CTLA4
ICOS
CTLA4
26.4
41.8
14.7
17.1
6.52
38.5
5.29
49.7
5.91
26.0
9.72
58.4
CD127
FAS
CD127
FAS

Figure 4

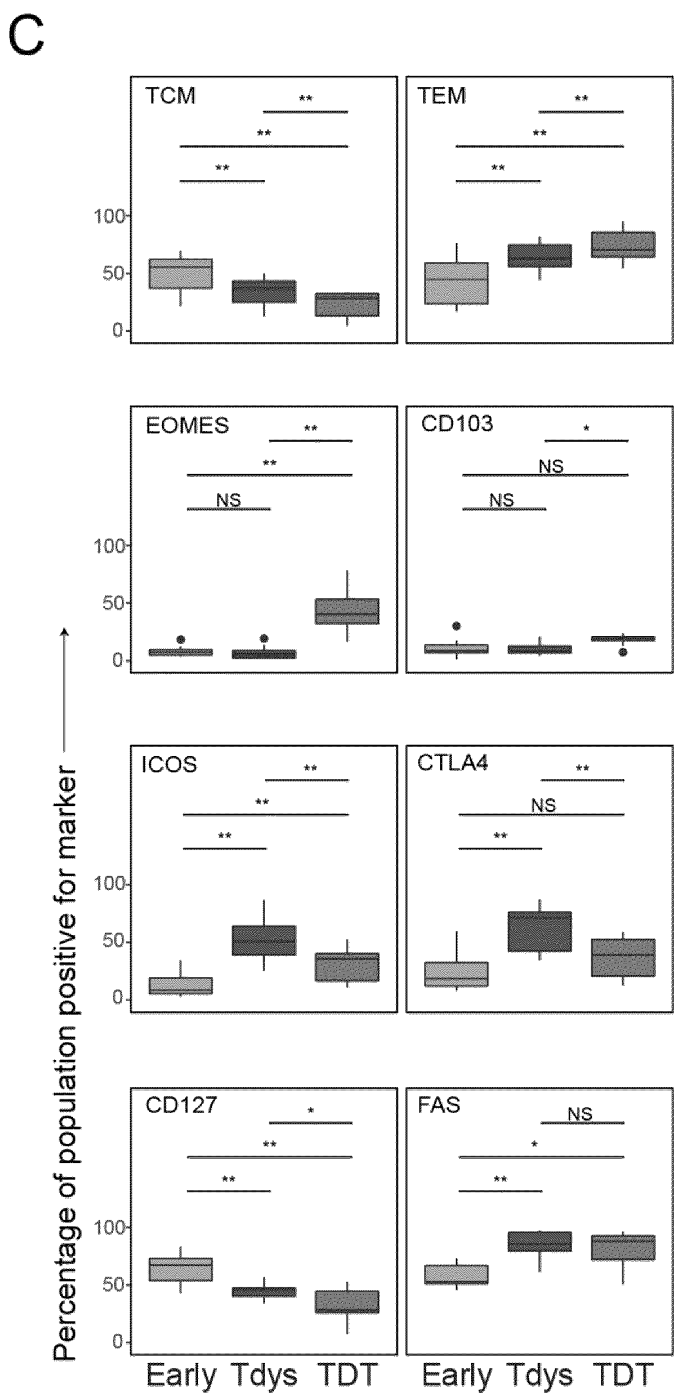

C
TCM
TEM
EOMES
CD103
ICOS
CTLA4
CD127
FAS
**
*
NS
100
50
0
Percentage of population positive for marker
Early Tdys TDT
Early Tdys TDT

Figure 4

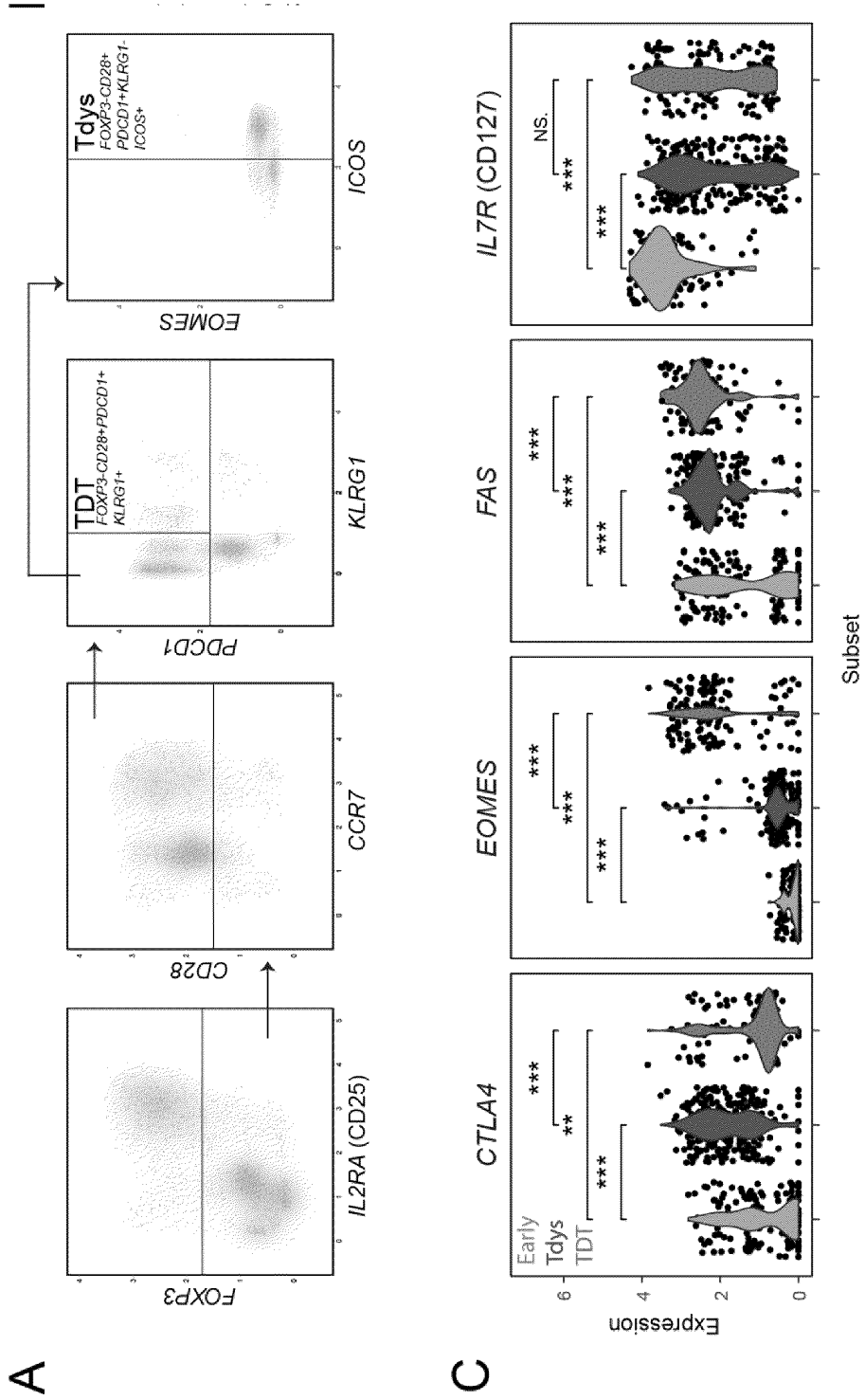

A
FOXP3
IL2RA (CD25)
CD28
CCR7
PDCD1
KLRG1
TDT
FOXP3-CD28+PDCD1+
KLRG1+
EOMES
ICOS
Tdys
FOXP3-CD28+
PDCD1+KLRG1-
ICOS+
C
CTLA4
EOMES
FAS
IL7R (CD127)
Early
Tdys
TDT
Expression
Subset
***
**
NS.

Figure 5

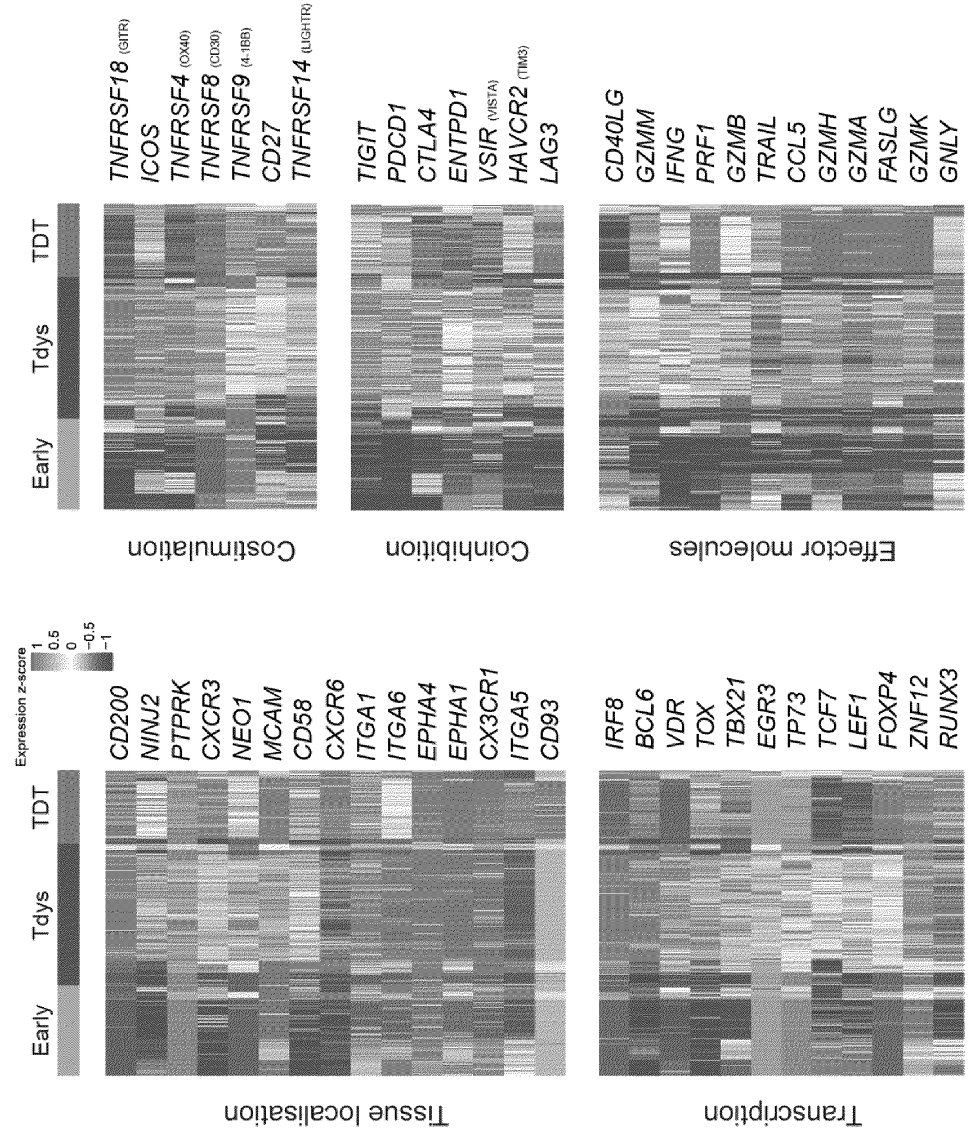


Figure 5 (continued)

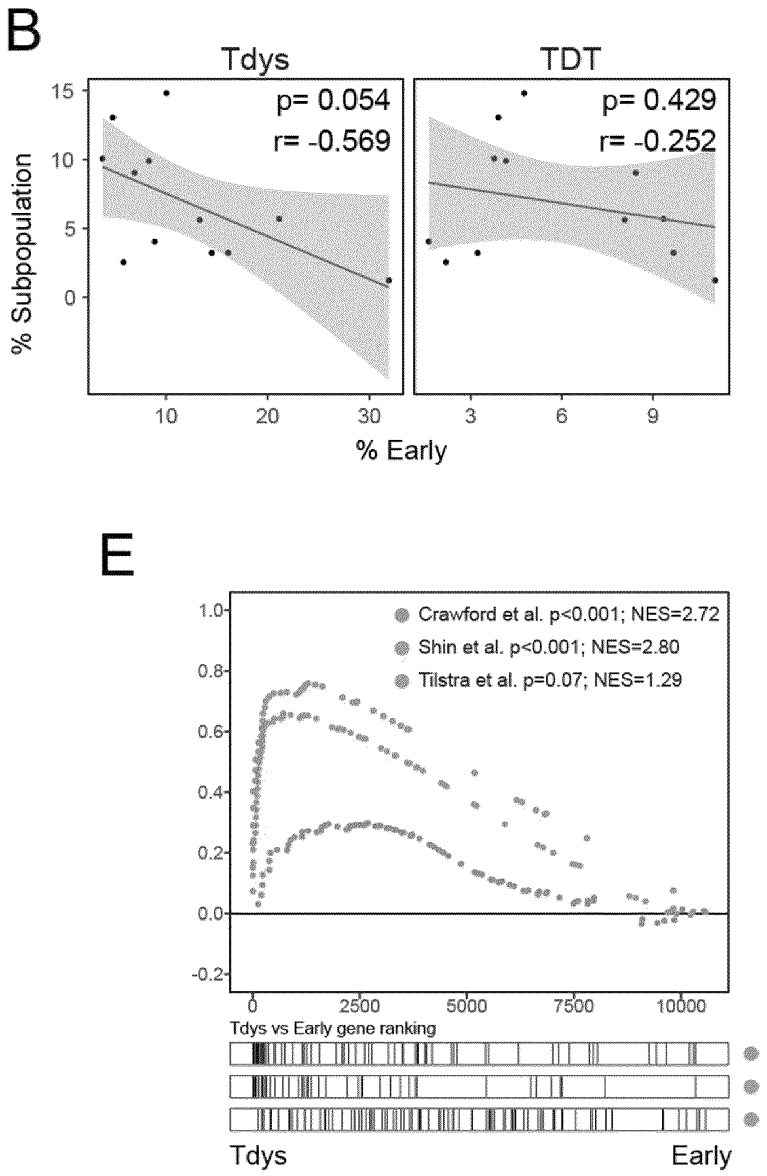


Figure 5 (continued)

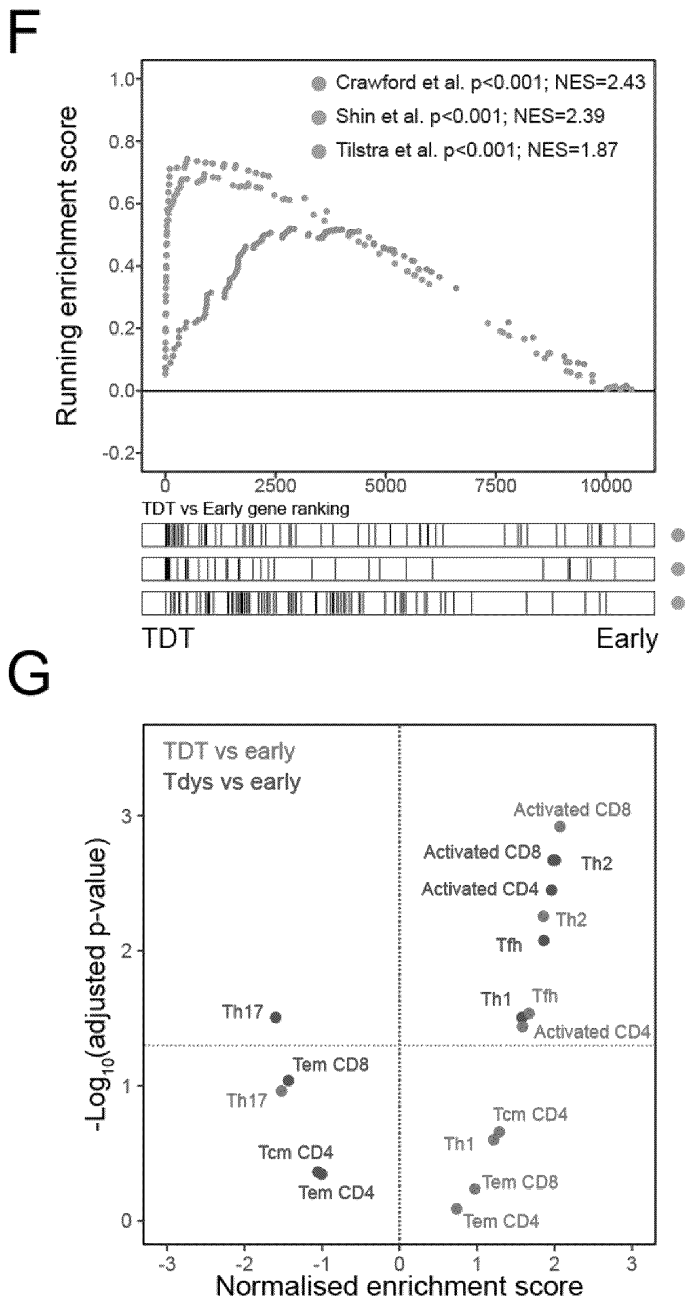


Figure 5 (continued)

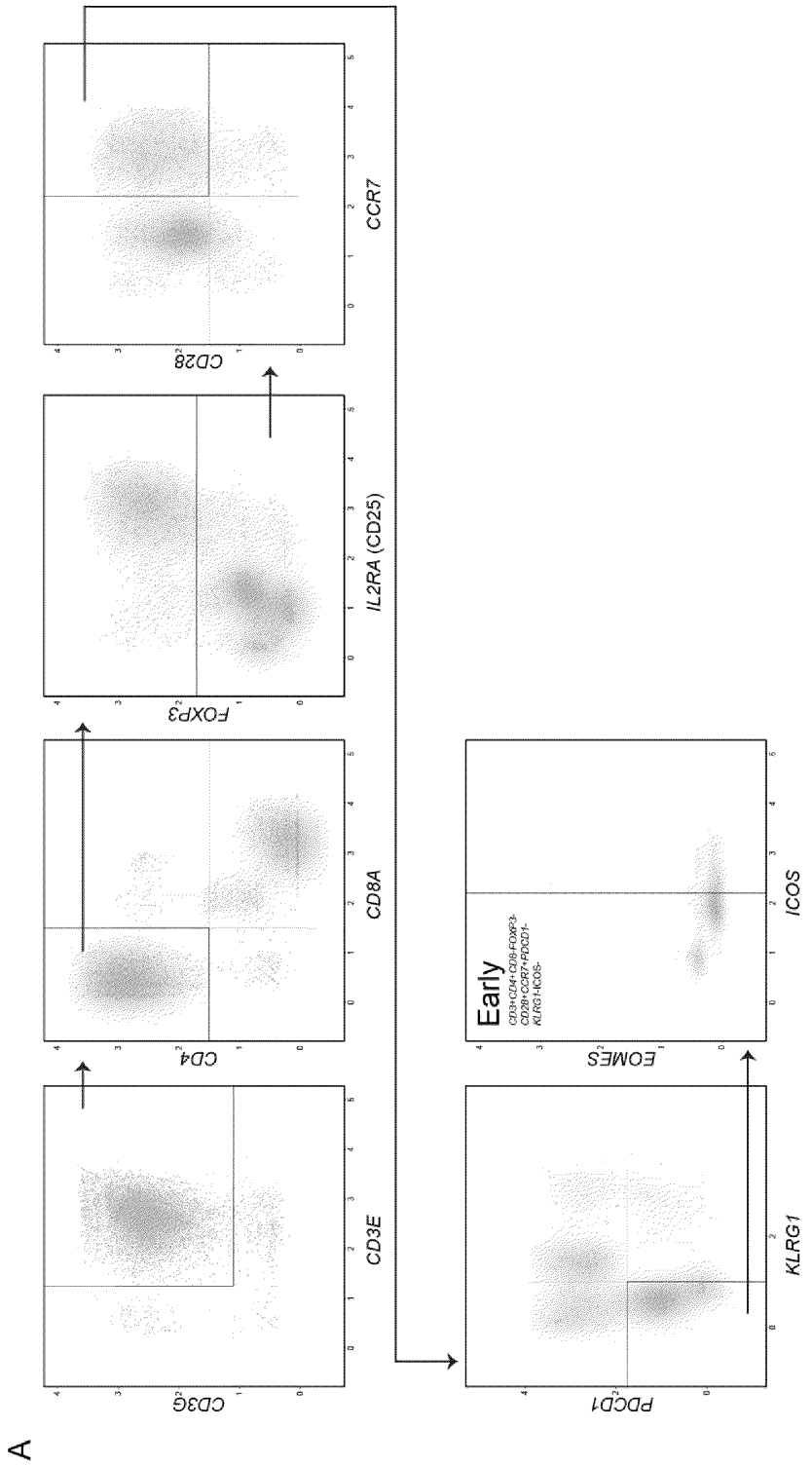

A
CD3G
CD3E
CD4
CD8A
FOXP3
IL2RA (CD25)
CD28
CCR7
PDCD1
KLRG1
EOMES
ICOS
Early
CD3+CD4+CD8-FOXP3-
CD28+CCR7+PDCD1-
KLRG1-ICOS-

Figure 6

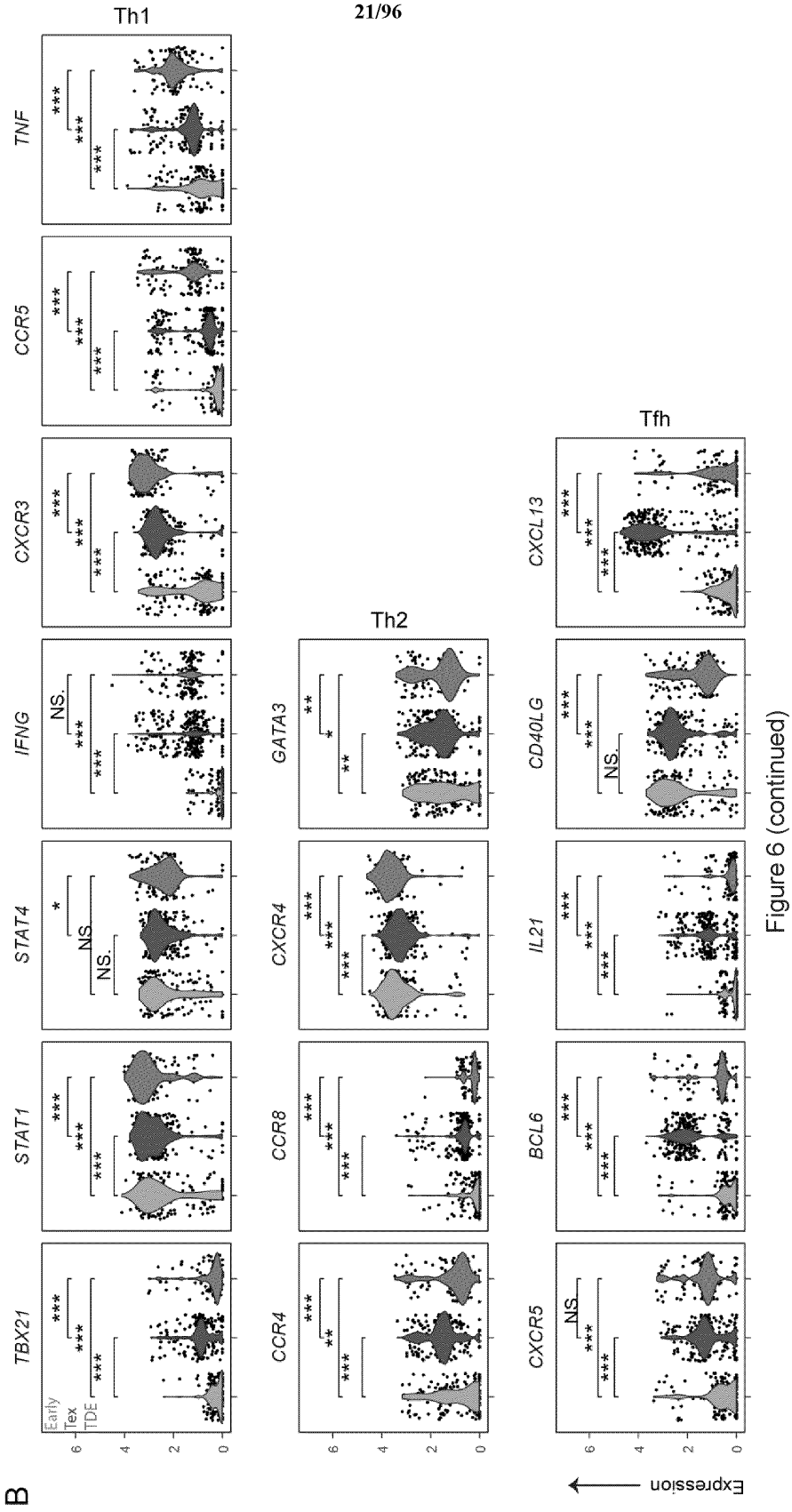


Figure 6 (continued)

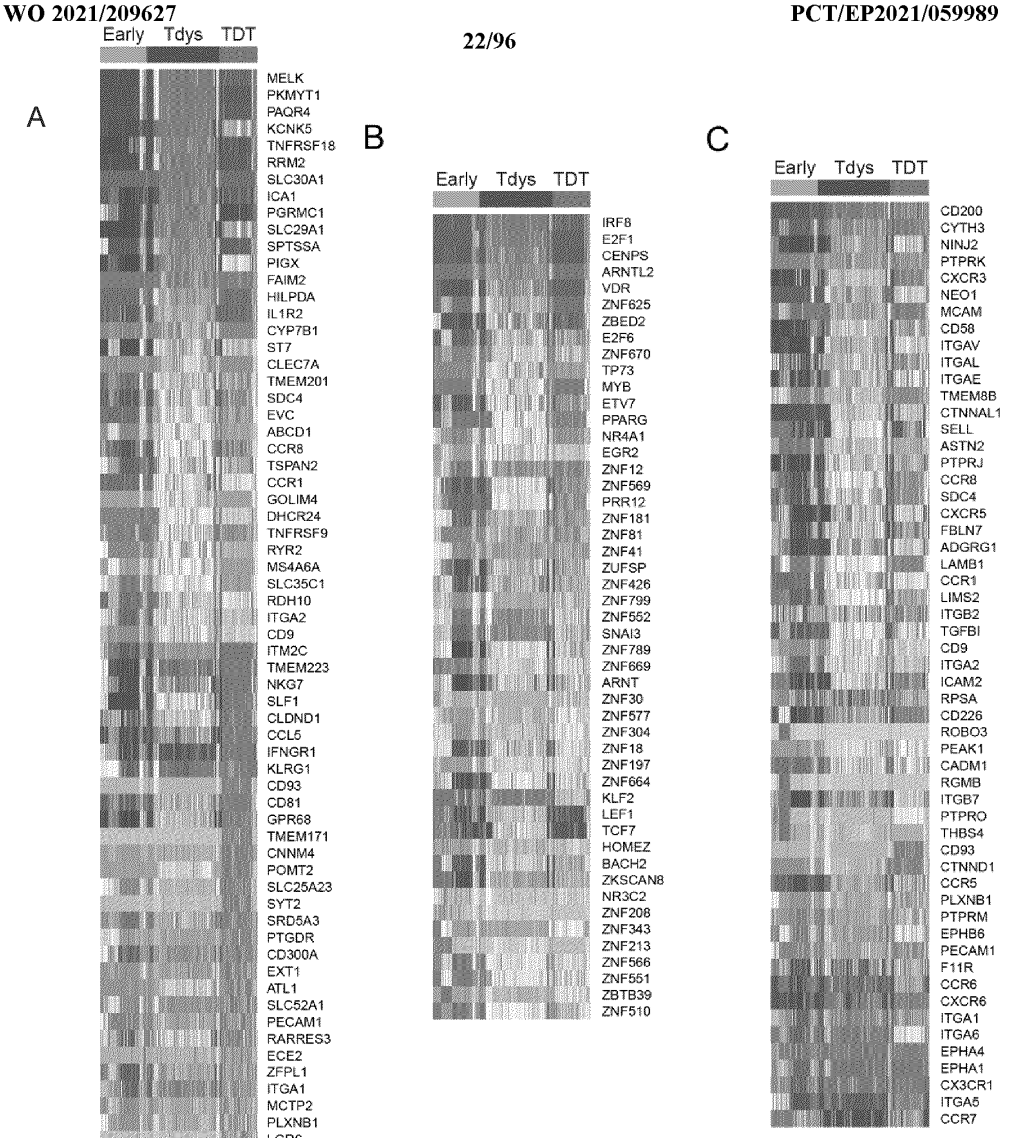

A
Early Tdys TDT
MELK
PKMYT1
PAQR4
KCNK5
TNFRSF18
RRM2
SLC30A1
ICA1
PGRMC1
SLC29A1
SPTSSA
PIGX
FAIM2
HILPDA
IL1R2
CYP7B1
ST7
CLEC7A
TMEM201
SDC4
EVC
ABCD1
CCR8
TSPAN2
CCR1
GOLIM4
DHCR24
TNFRSF9
RYR2
MS4A6A
SLC35C1
RDH10
ITGA2
CD9
ITM2C
TMEM223
NKG7
SLF1
CLDND1
CCL5
IFNGR1
KLRG1
CD93
CD81
GPR68
TMEM171
CNNM4
POMT2
SLC25A23
SYT2
SRD5A3
PTGDR
CD300A
EXT1
ATL1
SLC52A1
PECAM1
RARRES3
ECE2
ZFPL1
ITGA1
MCTP2
PLXNB1
LGR6
ARHGAP26
IGF2R
ATP1A3
STRN4
PTPRM
FSD1L
PIGA
TMEM167B
SLC35F5
EXTL3
VANGL1
SCAMP1
GPAM
ADAM28
MS4A1
TP53I11
CCRL2
DPY19L4
C1orf162
AQP3
SELL
CACNA1I
IL11RA
KCTD7
SSBP2
ABCB1
SLC25A51
ATG9B
GPR15
ST3GAL2
SLC8B1
RGMB
GAL3ST4
F2RL1
NCKIPSD
FAM171A1
SLC2A11
GCLC
B
Early Tdys TDT
IRF8
E2F1
CENPS
ARNTL2
VDR
ZNF625
ZBED2
E2F6
ZNF670
TP73
MYB
ETV7
PPARG
NR4A1
EGR2
ZNF12
ZNF569
PRR12
ZNF181
ZNF81
ZNF41
ZUFSP
ZNF426
ZNF799
ZNF552
SNAI3
ZNF789
ZNF669
ARNT
ZNF30
ZNF577
ZNF304
ZNF18
ZNF197
ZNF664
KLF2
LEF1
TCF7
HOMEZ
BACH2
ZKSCAN8
NR3C2
ZNF208
ZNF343
ZNF213
ZNF566
ZNF551
ZBTB39
ZNF510
C
Early Tdys TDT
CD200
CYTH3
NINJ2
PTPRK
CXCR3
NEO1
MCAM
CD58
ITGAV
ITGAL
ITGAE
TMEM8B
CTNNAL1
SELL
ASTN2
PTPRJ
CCR8
SDC4
CXCR5
FBLN7
ADGRG1
LAMB1
CCR1
LIMS2
ITGB2
TGFBI
CD9
ITGA2
ICAM2
RPSA
CD226
ROBO3
PEAK1
CADM1
RGMB
ITGB7
PTPRO
THBS4
CD93
CTNND1
CCR5
PLXNB1
PTPRM
EPHB6
PECAM1
F11R
CCR6
CXCR6
ITGA1
ITGA6
EPHA4
EPHA1
CX3CR1
ITGA5
CCR7

Figure 7

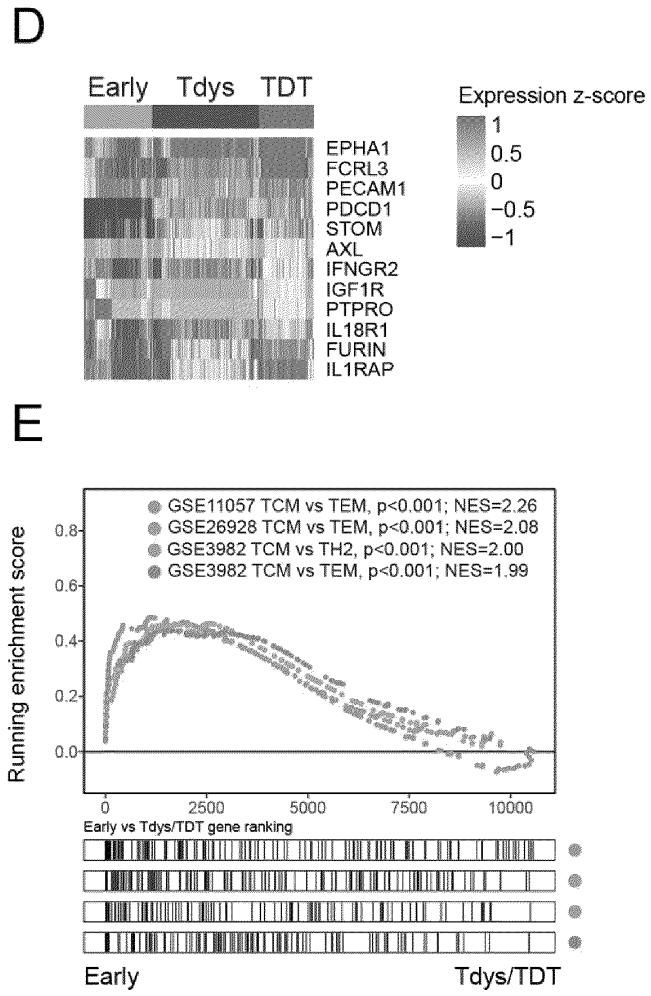


Figure 7 (continued)

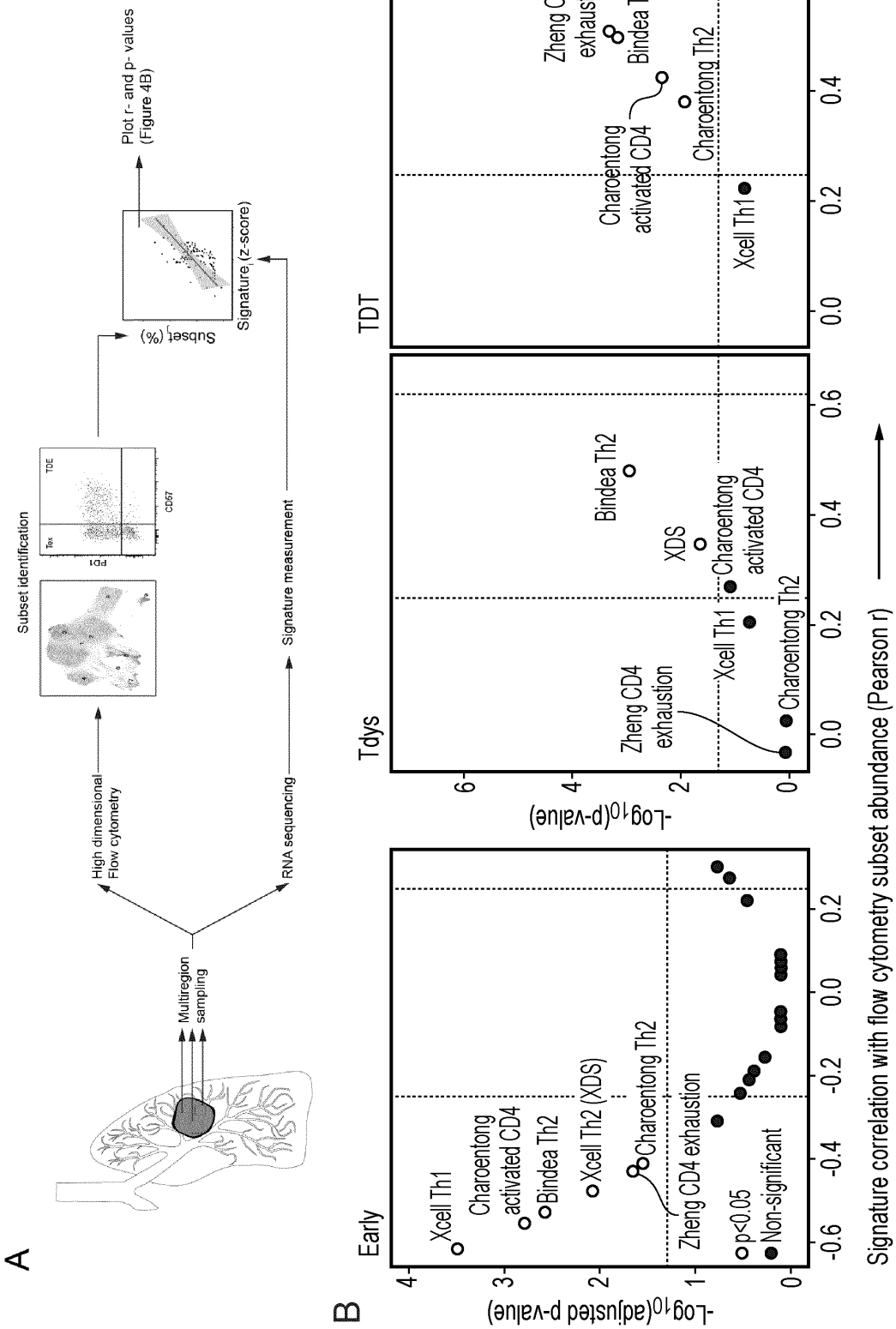

A
Multiregion sampling
High dimensional Flow cytometry
RNA sequencing
Subset identification
Subset (%)
Plot r- and p- values (Figure 4B)
Signature (z-score)
Signature measurement
B
Early
TDT
Tdys
-Log10(adjusted p-value)
-Log10(p-value)
Xcell Th1
Charoentong activated CD4
Bindea Th2
Xcell Th2 (XDS)
Charoentong Th2
Zheng CD4 exhaustion
p<0.05
Non-significant
XDS
Signature correlation with flow cytometry subset abundance (Pearson r)

FIG. 8

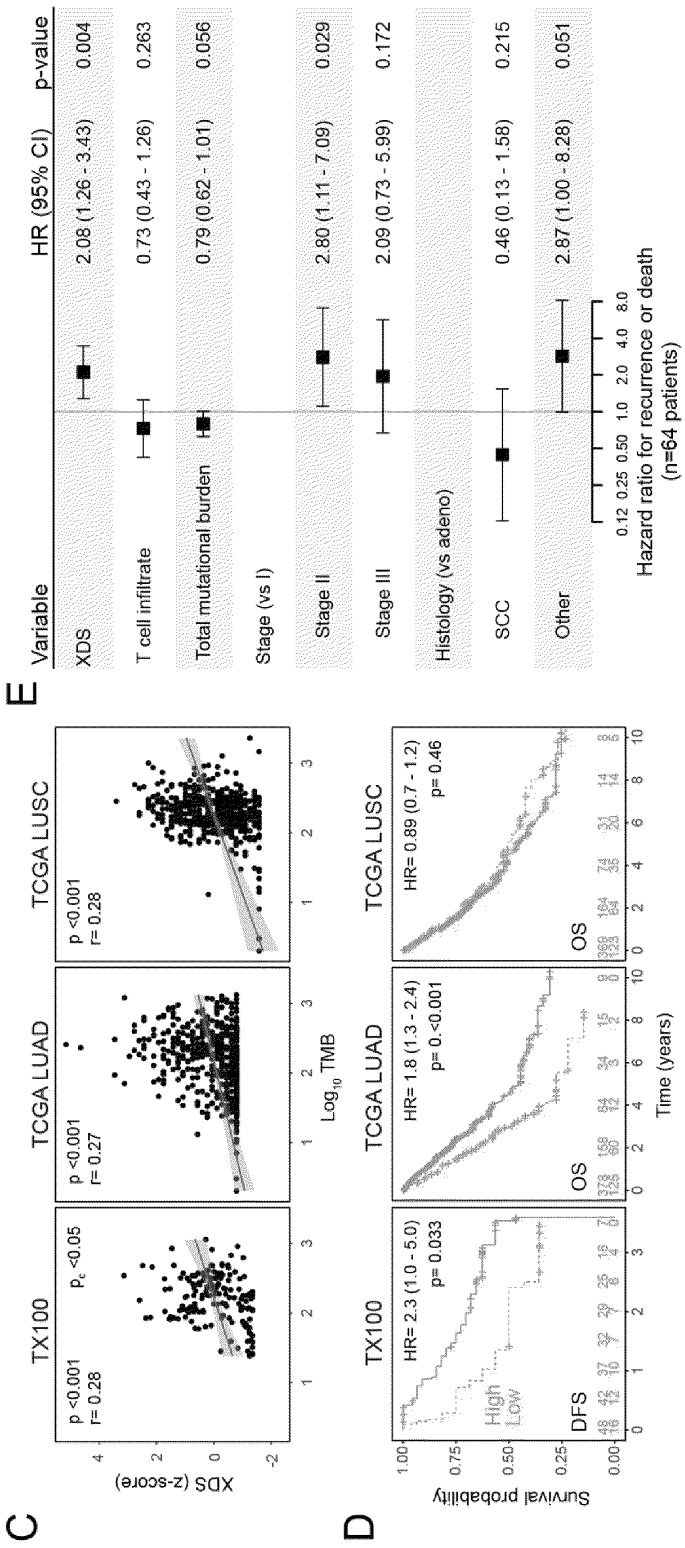


Figure 8 (continued)

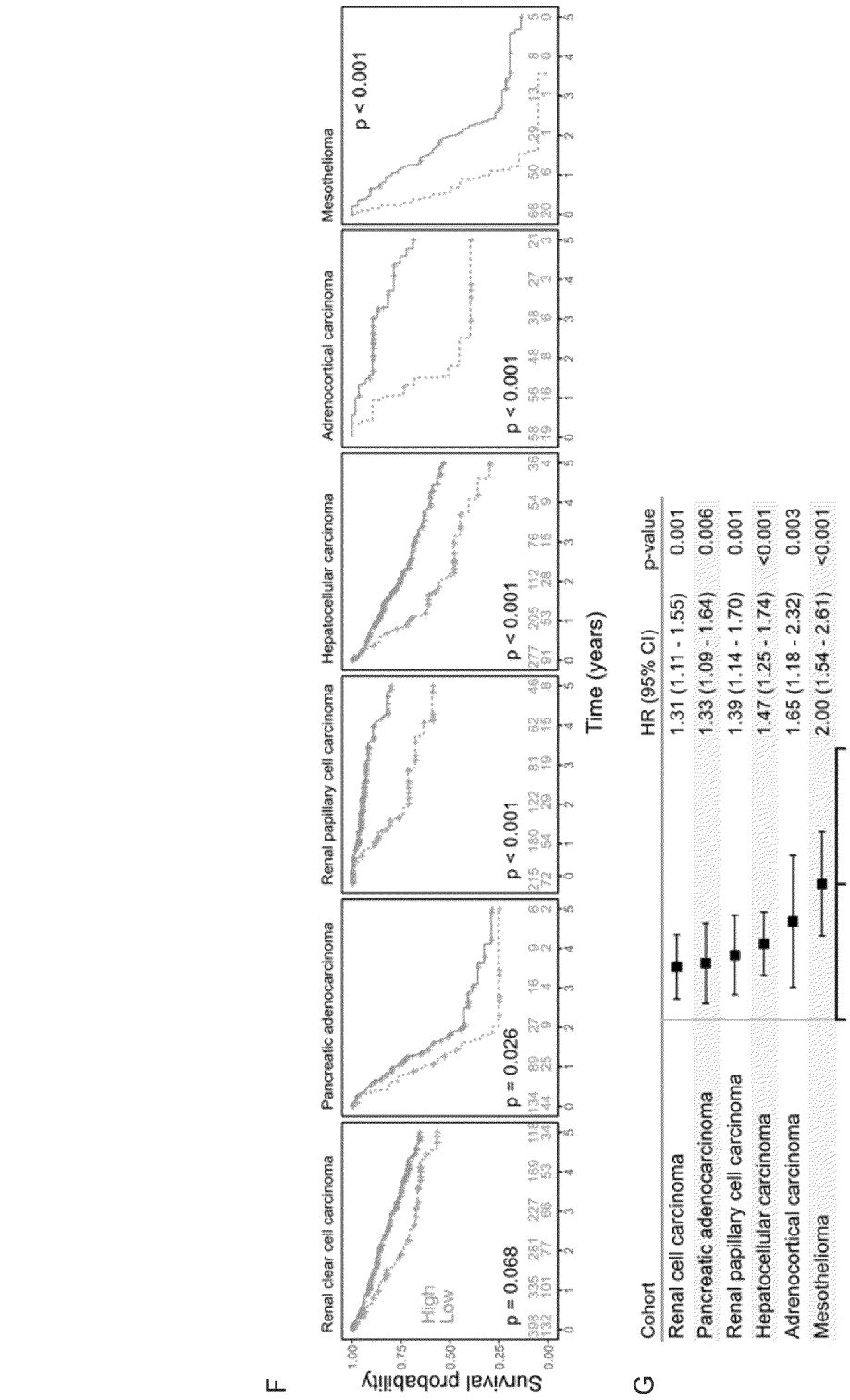
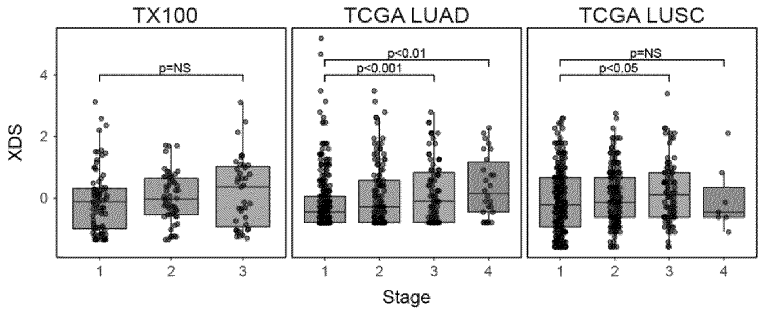


Figure 8 (continued)

A


TX100
TCGA LUAD
TCGA LUSC
p=NS
p<0.01
p<0.001
p=NS
p<0.05
XDS
4
2
0
1
2
3
1
2
3
4
1
2
3
4
Stage

B

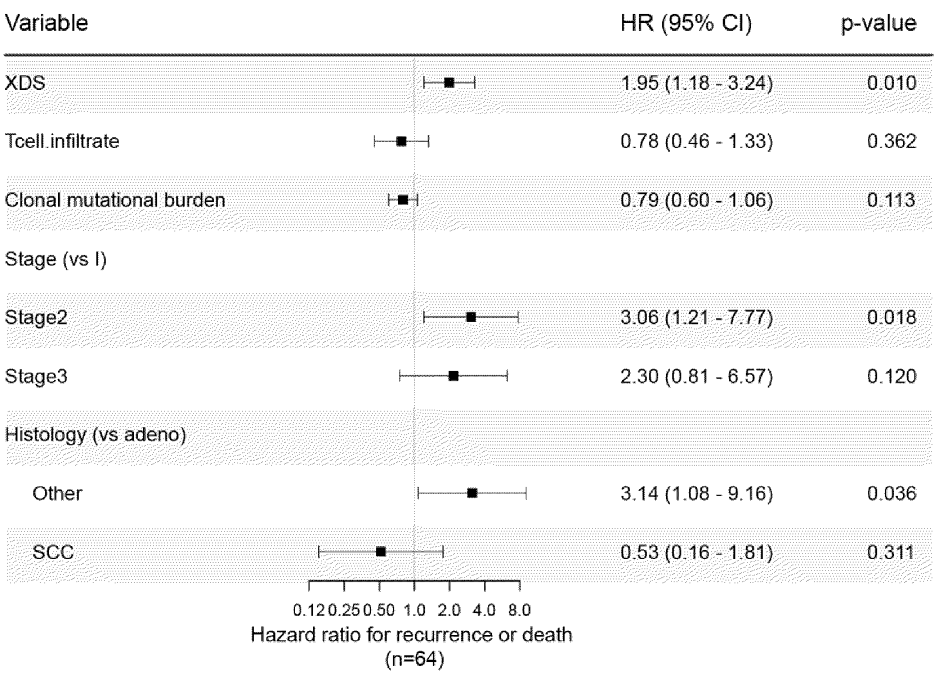

Variable
HR (95% CI)
p-value
XDS
1.95 (1.18 - 3.24)
0.010
Tcell.infiltrate
0.78 (0.46 - 1.33)
0.362
Clonal mutational burden
0.79 (0.60 - 1.06)
0.113
Stage (vs I)
Stage2
3.06 (1.21 - 7.77)
0.018
Stage3
2.30 (0.81 - 6.57)
0.120
Histology (vs adeno)
Other
3.14 (1.08 - 9.16)
0.036
SCC
0.53 (0.16 - 1.81)
0.311
0.12 0.25 0.50 1.0 2.0 4.0 8.0
Hazard ratio for recurrence or death
(n=64)

Figure 9

C

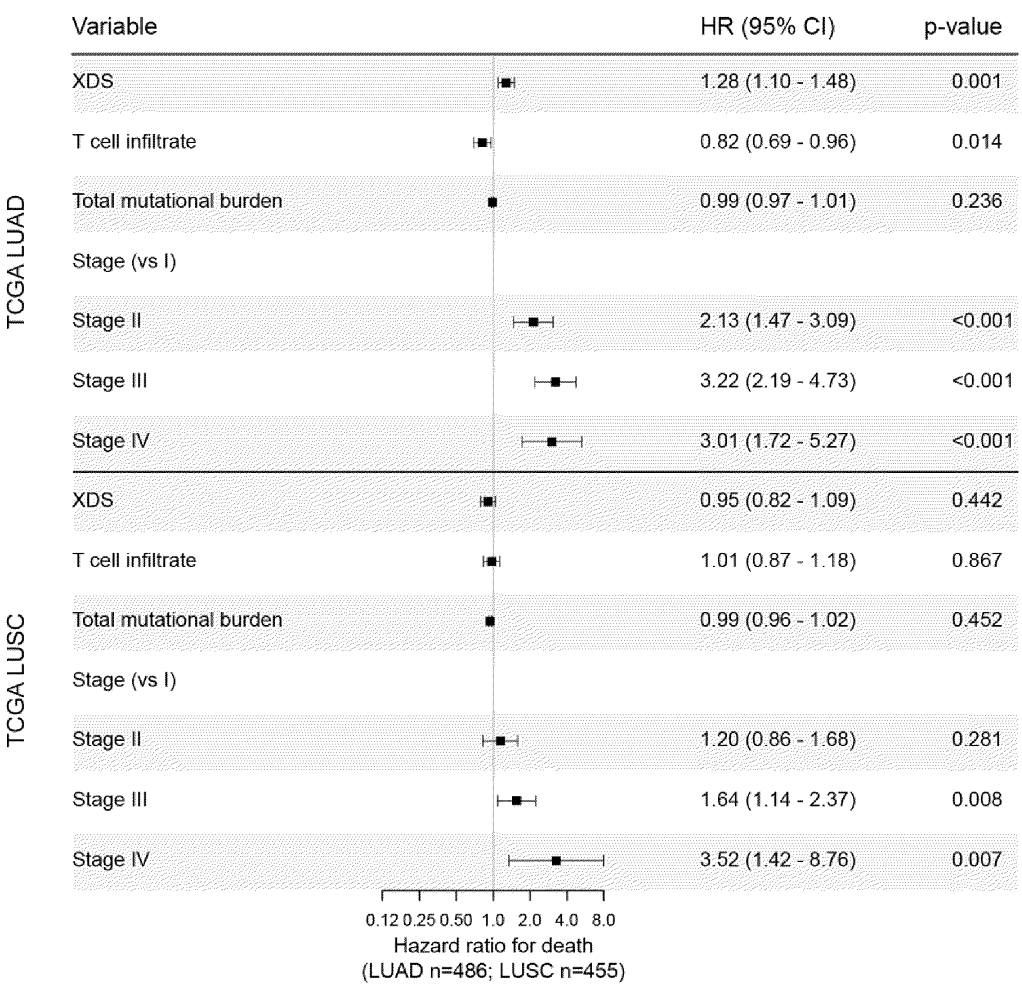


Figure 9 (continued)

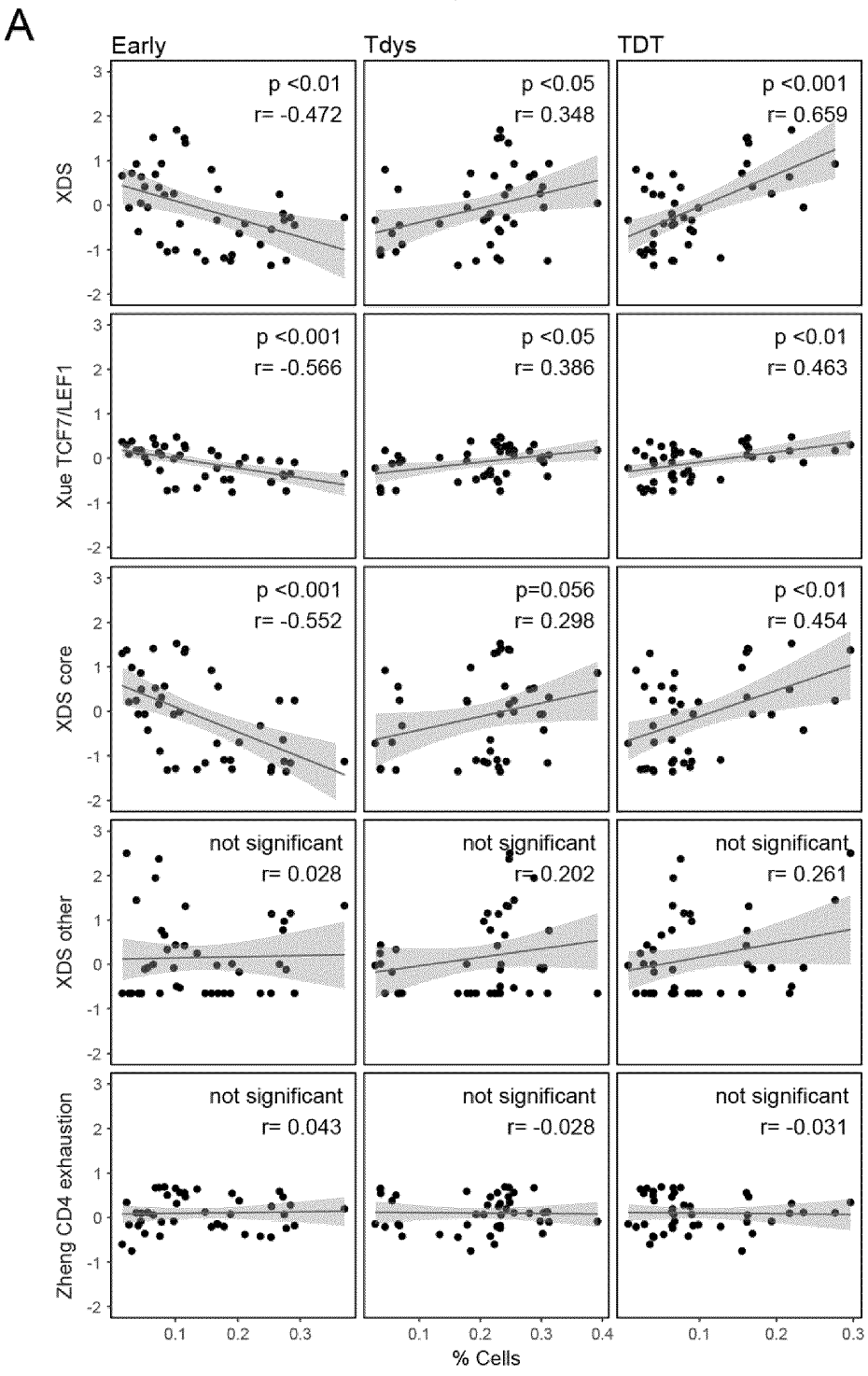

A
Early
Tdys
TDT
XDS
Xue TCF7/LEF1
XDS core
XDS other
Zheng CD4 exhaustion
p <0.01
r= -0.472
p <0.05
r= 0.348
p <0.001
r= 0.659
p <0.001
r= -0.566
p <0.05
r= 0.386
p <0.01
r= 0.463
p <0.001
r= -0.552
p=0.056
r= 0.298
p <0.01
r= 0.454
not significant
r= 0.028
not significant
r= 0.202
not significant
r= 0.261
not significant
r= 0.043
not significant
r= -0.028
not significant
r= -0.031
% Cells

Figure 10

B

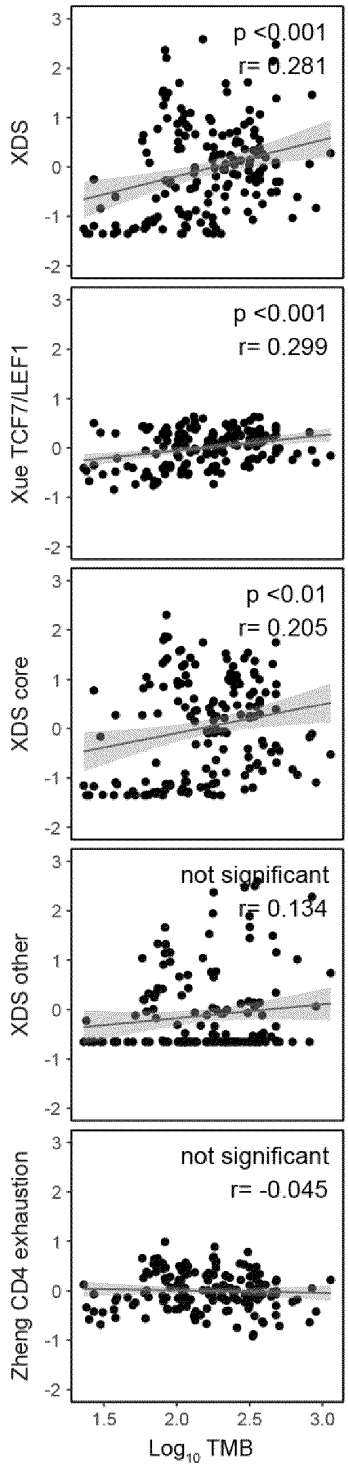

p <0.001
r= 0.281
XDS
p <0.001
r= 0.299
Xue TCF7/LEF1
p <0.01
r= 0.205
XDS core
not significant
r= 0.134
XDS other
not significant
r= -0.045
Zheng CD4 exhaustion
1.5
2.0
2.5
3.0
Log10 TMB

Figure 10

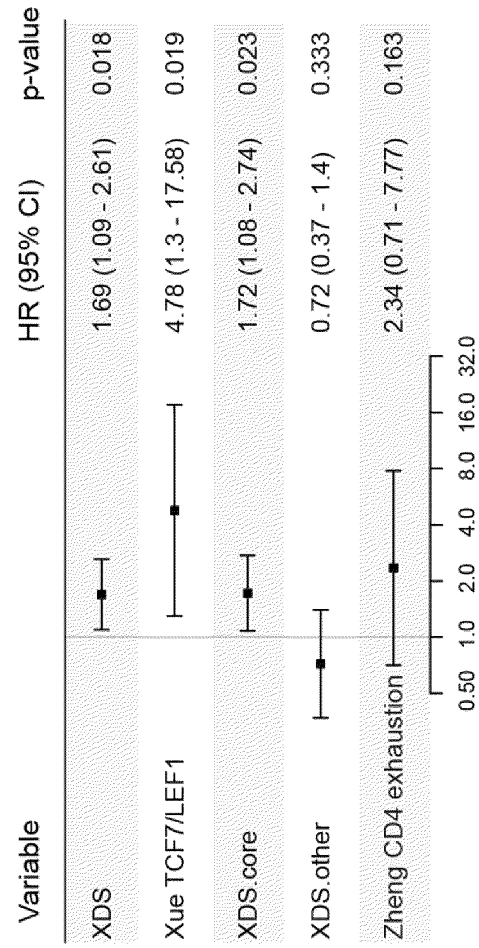


Figure 10 (continued)

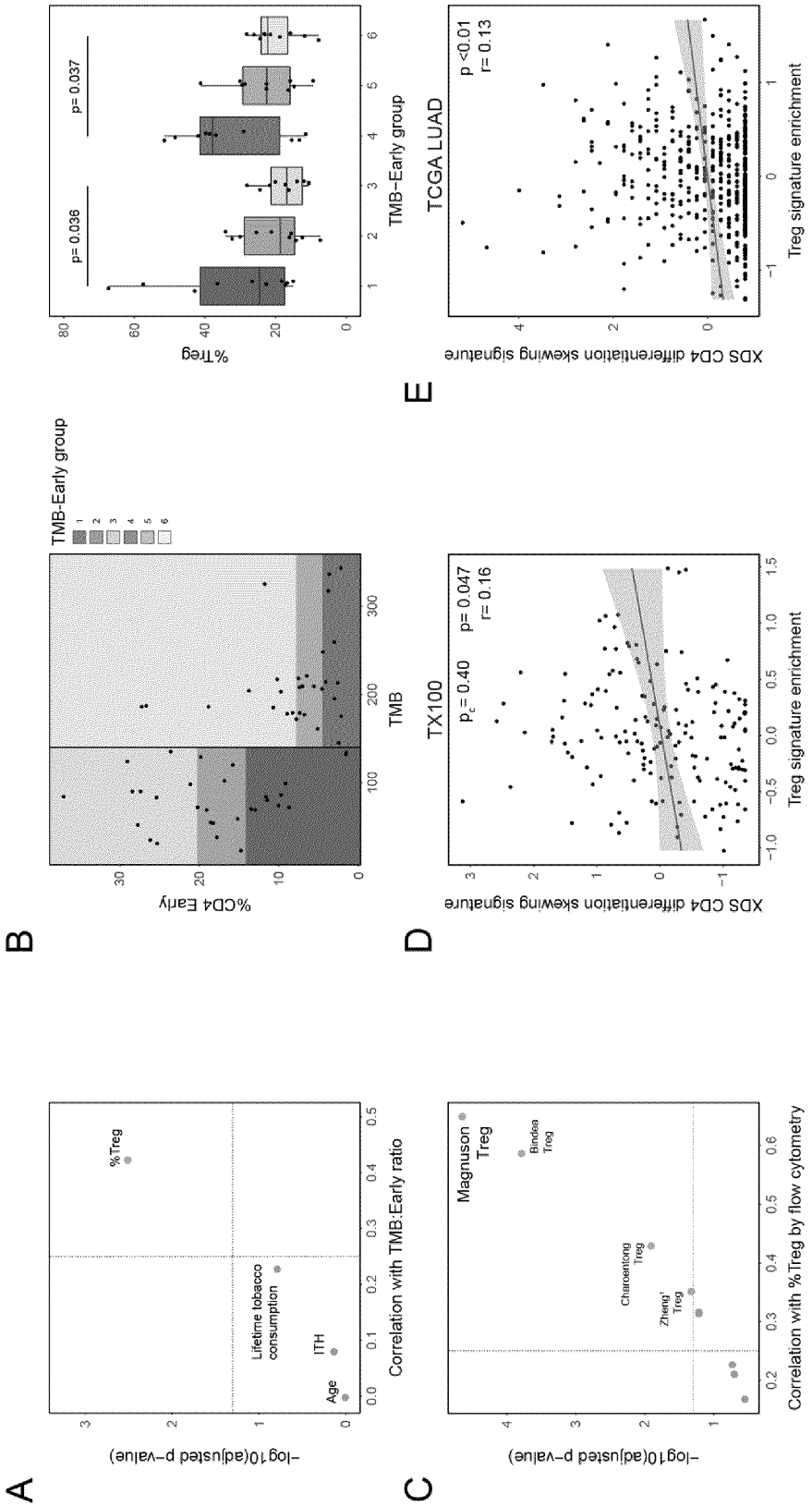

A
−log10(adjusted p−value)
%Treg
Lifetime tobacco consumption
ITH
Age
0.0 0.1 0.2 0.3 0.4 0.5
Correlation with TMB:Early ratio
B
%CD4 Early
TMB
TMB-Early group
1 2 3 4 5 6
C
−log10(adjusted p−value)
Magnuson Treg
Bindea Treg
Charoentong Treg
Zheng' Treg
0.2 0.3 0.4 0.5 0.6
Correlation with %Treg by flow cytometry
D
XDS CD4 differentiation skewing signature
TX100
p_c = 0.40 p= 0.047
r= 0.16
Treg signature enrichment
E
%Treg
p= 0.036 p= 0.037
TMB−Early group
XDS CD4 differentiation skewing signature
TCGA LUAD
p <0.01
r= 0.13
Treg signature enrichment

Figure 11

F

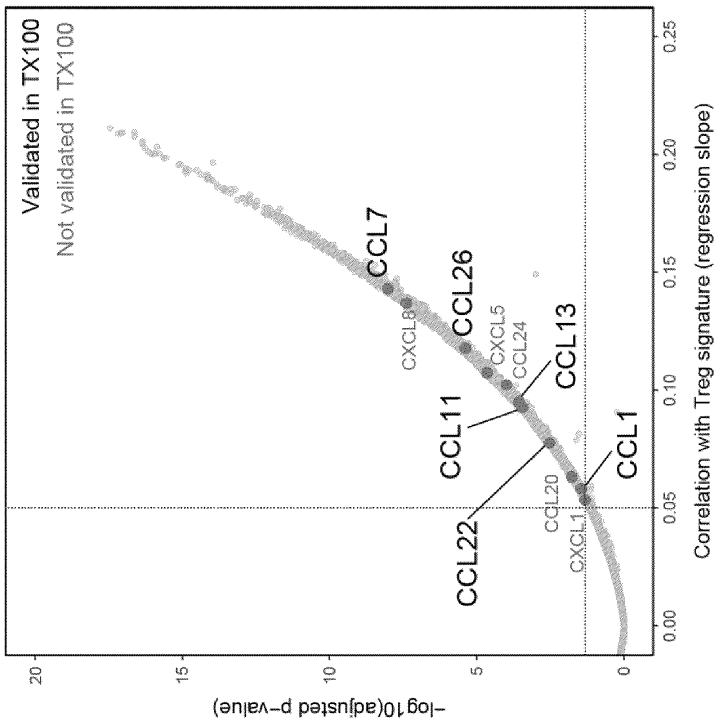


Figure 11 (continued)

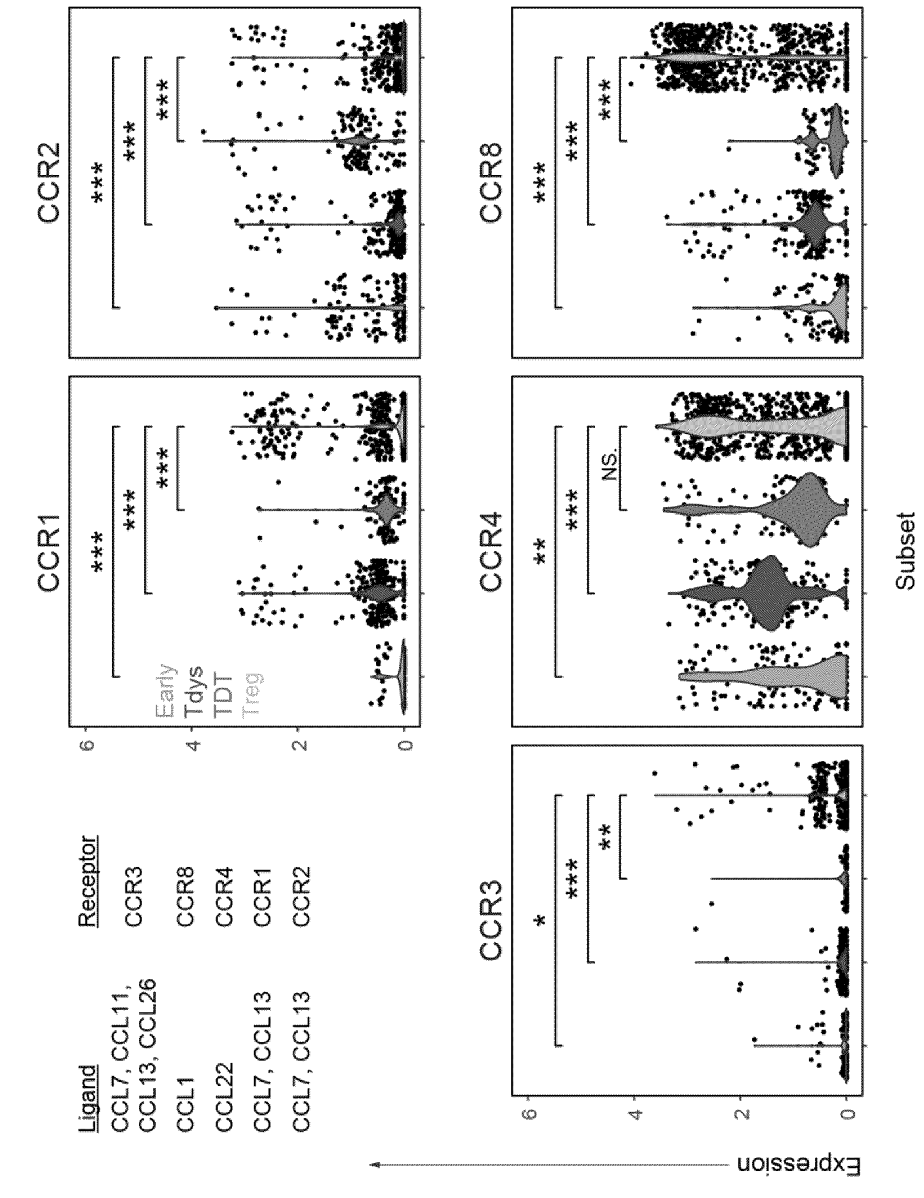
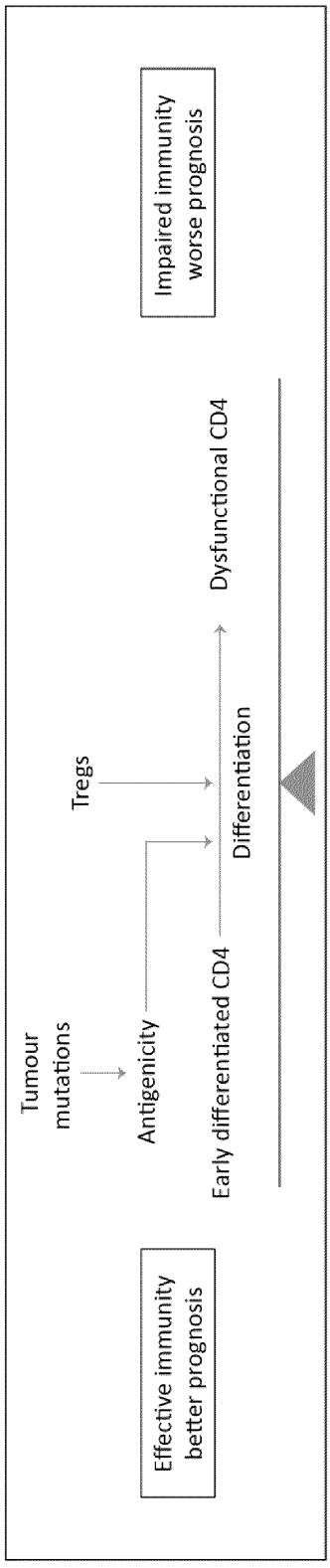


Figure 11 (continued)



H

Figure 11 (continued)

A

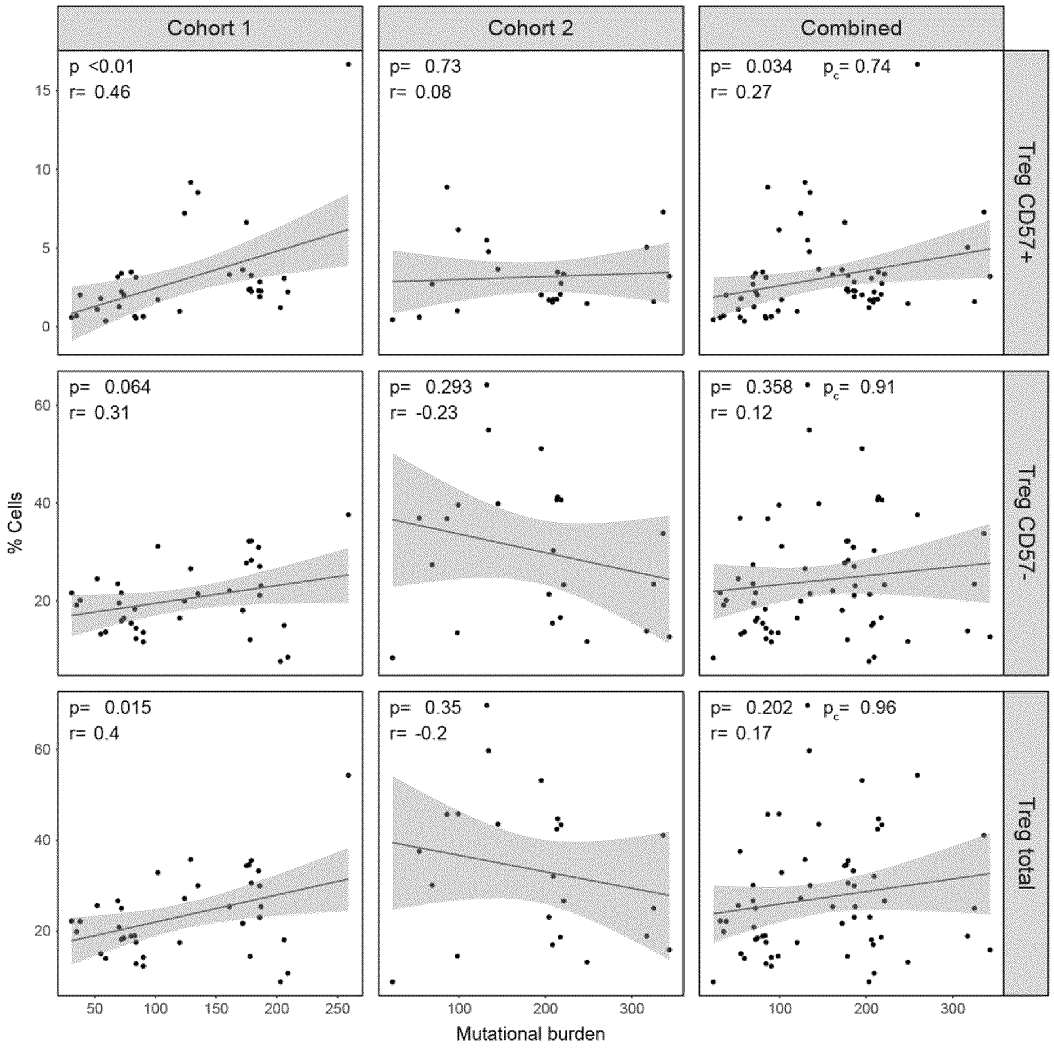

Cohort 1
Cohort 2
Combined
Treg CD57+
Treg CD57-
Treg total
p <0.01
r= 0.46
p= 0.73
r= 0.08
p= 0.034
p_c= 0.74
r= 0.27
p= 0.064
r= 0.31
p= 0.293
r= -0.23
p= 0.358
p_c= 0.91
r= 0.12
p= 0.015
r= 0.4
p= 0.35
r= -0.2
p= 0.202
p_c= 0.96
r= 0.17
% Cells
Mutational burden

Figure 12

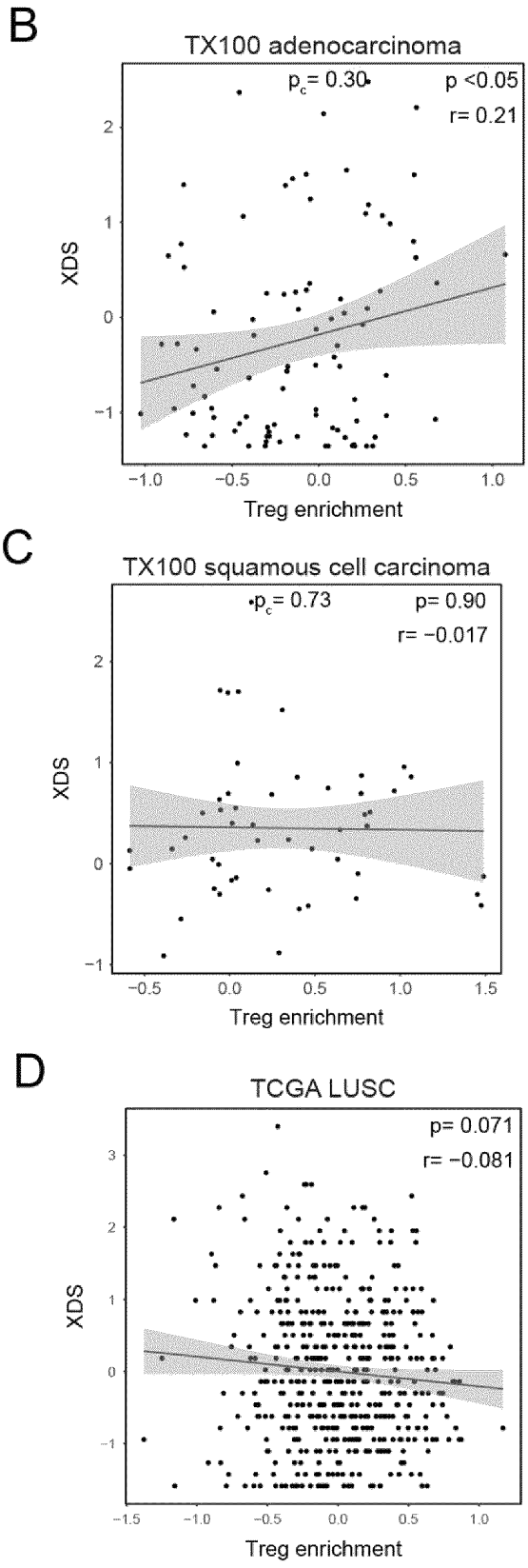
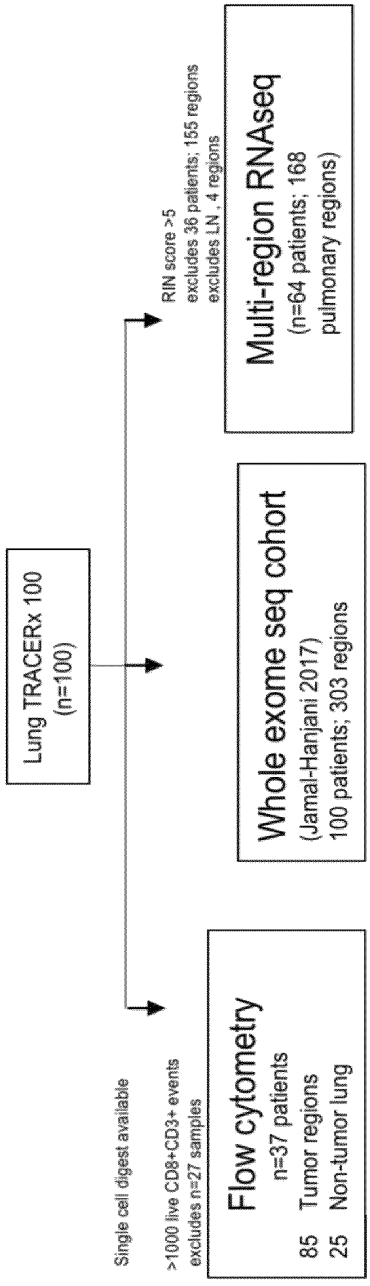

B
TX100 adenocarcinoma
p_c= 0.30
p <0.05
r= 0.21
XDS
Treg enrichment
C
TX100 squamous cell carcinoma
p_c= 0.73
p= 0.90
r= −0.017
XDS
Treg enrichment
D
TCGA LUSC
p= 0.071
r= −0.081
XDS
Treg enrichment

Figure 12

a



c

Histology	Patients	Tumor regions	Non-tumor lung
Total	37	85	25
LUAD	17	34	10
LUSC	18	39	12
Other	3	13	2

Figure 13

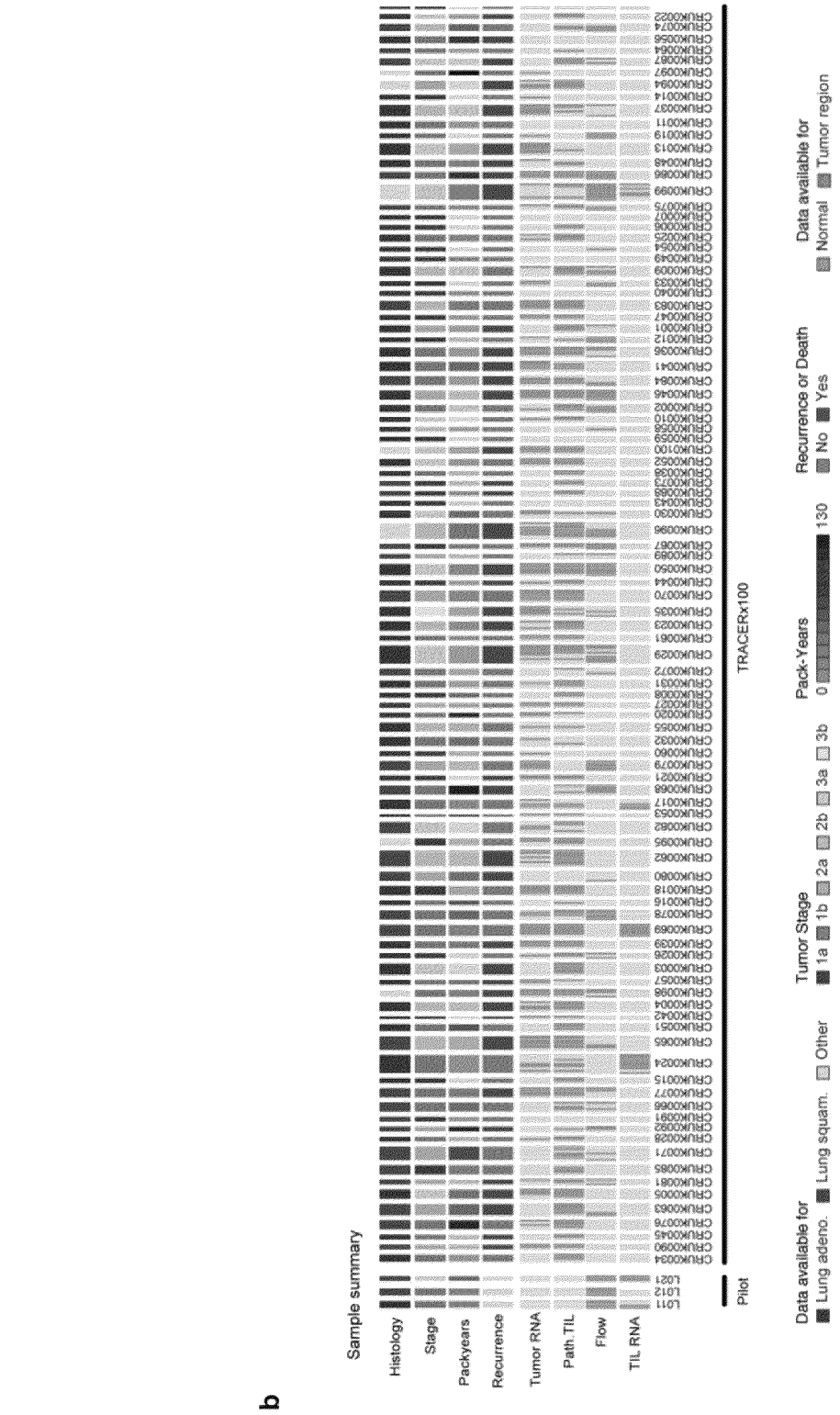


Figure 13 (continued)

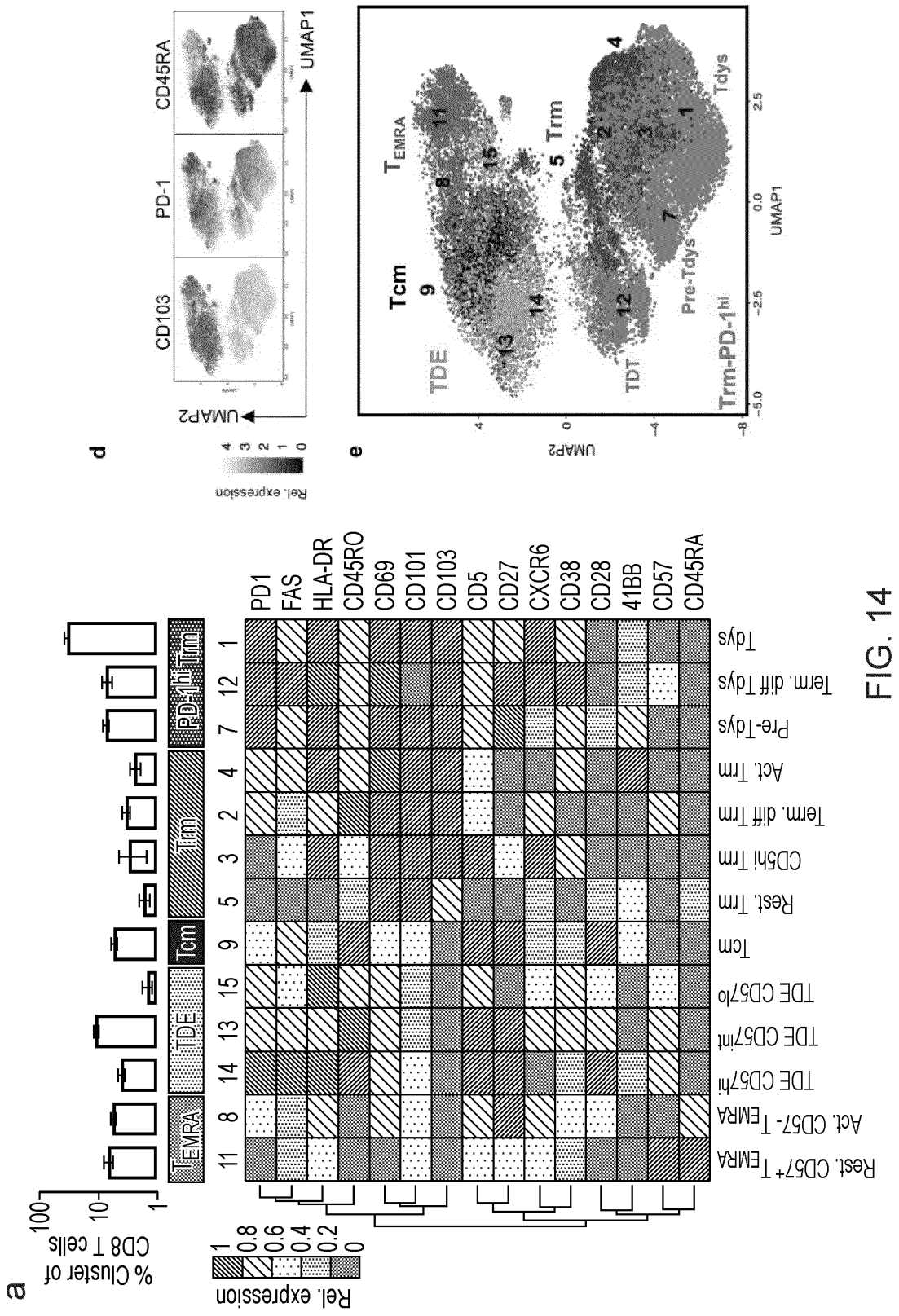

a
% Cluster of CD8 T cells
100
10
1
TEMRA
TDE
Tcm
Trm
PD-1hi Trm
11 8 14 13 15 9 5 2 3 4 7 12 1
Rel. expression
1 0.8 0.6 0.4 0.2 0
PD1
FAS
HLA-DR
CD45RO
CD69
CD101
CD103
CD5
CD27
CXCR6
CD38
CD28
41BB
CD57
CD45RA
Rest. CD57+ TEMRA
Act. CD57- TEMRA
TDE CD57hi
TDE CD57int
TDE CD57lo
Tcm
Rest. Trm
CD5hi Trm
Term. diff Trm
Act. Trm
Pre-Tdys
Term. diff Tdys
Tdys
d
CD103
PD-1
CD45RA
UMAP2
UMAP1
Rel. expression
4 3 2 1 0
e
Tcm
9
TEMRA
11
8
15
TDE
13
14
5 Trm
2
4
3
1
12
TDT
Pre-Tdys
7
Trm-PD-1hi
Tdys
UMAP2
UMAP1
4
0
−4
−8
−5.0
−2.5
0.0
2.5

FIG. 14

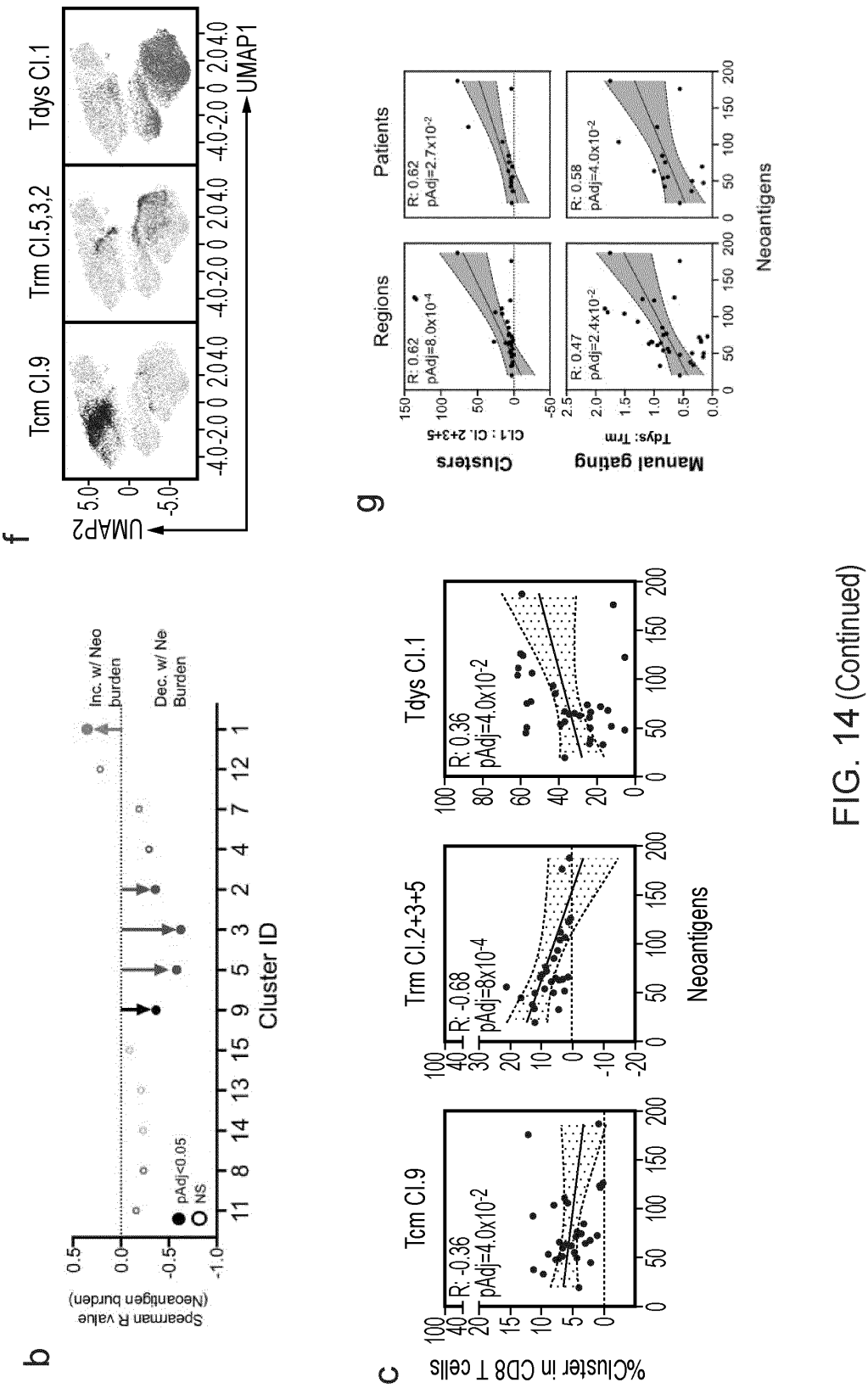


FIG. 14 (Continued)

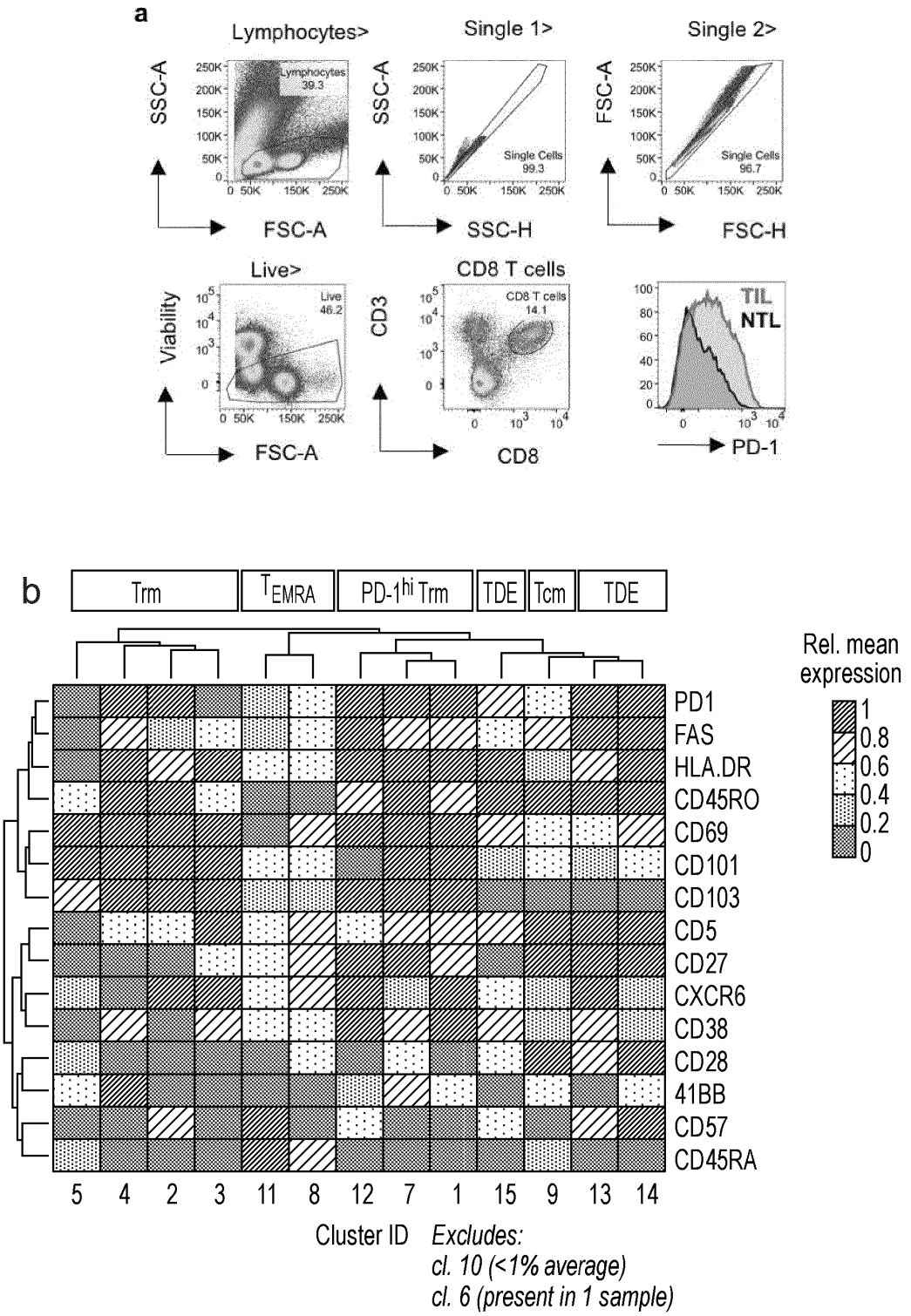

a
Lymphocytes>
Single 1>
Single 2>
SSC-A
FSC-A
Lymphocytes
39.3
SSC-H
Single Cells
99.3
FSC-H
Single Cells
96.7
Live>
CD8 T cells
Viability
Live
46.2
CD3
CD8 T cells
14.1
CD8
TIL
NTL
PD-1
b
Trm
TEMRA
PD-1hi Trm
TDE
Tcm
TDE
Rel. mean expression
1
0.8
0.6
0.4
0.2
0
PD1
FAS
HLA.DR
CD45RO
CD69
CD101
CD103
CD5
CD27
CXCR6
CD38
CD28
41BB
CD57
CD45RA
5 4 2 3 11 8 12 7 1 15 9 13 14
Cluster ID
Excludes:
cl. 10 (<1% average)
cl. 6 (present in 1 sample)

FIG. 15

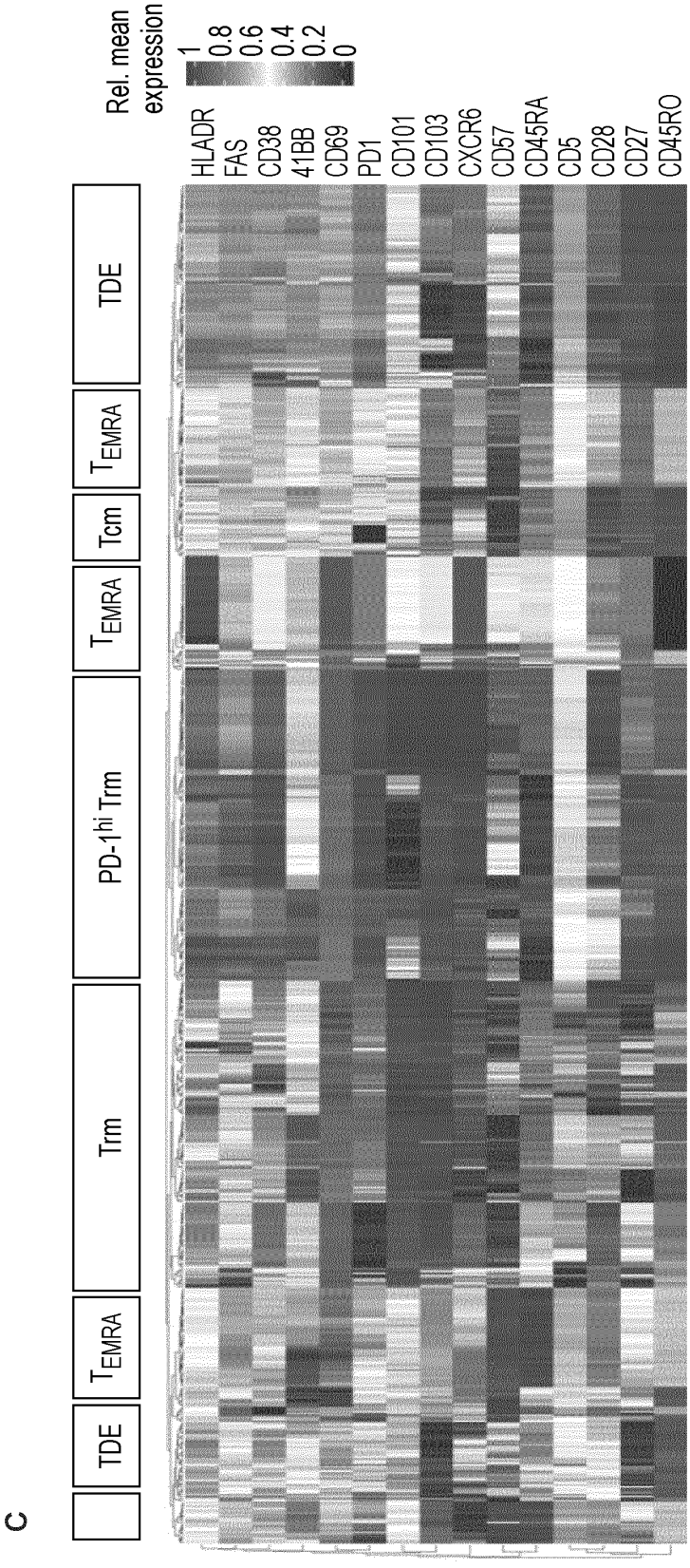


FIG. 15 (Continued)

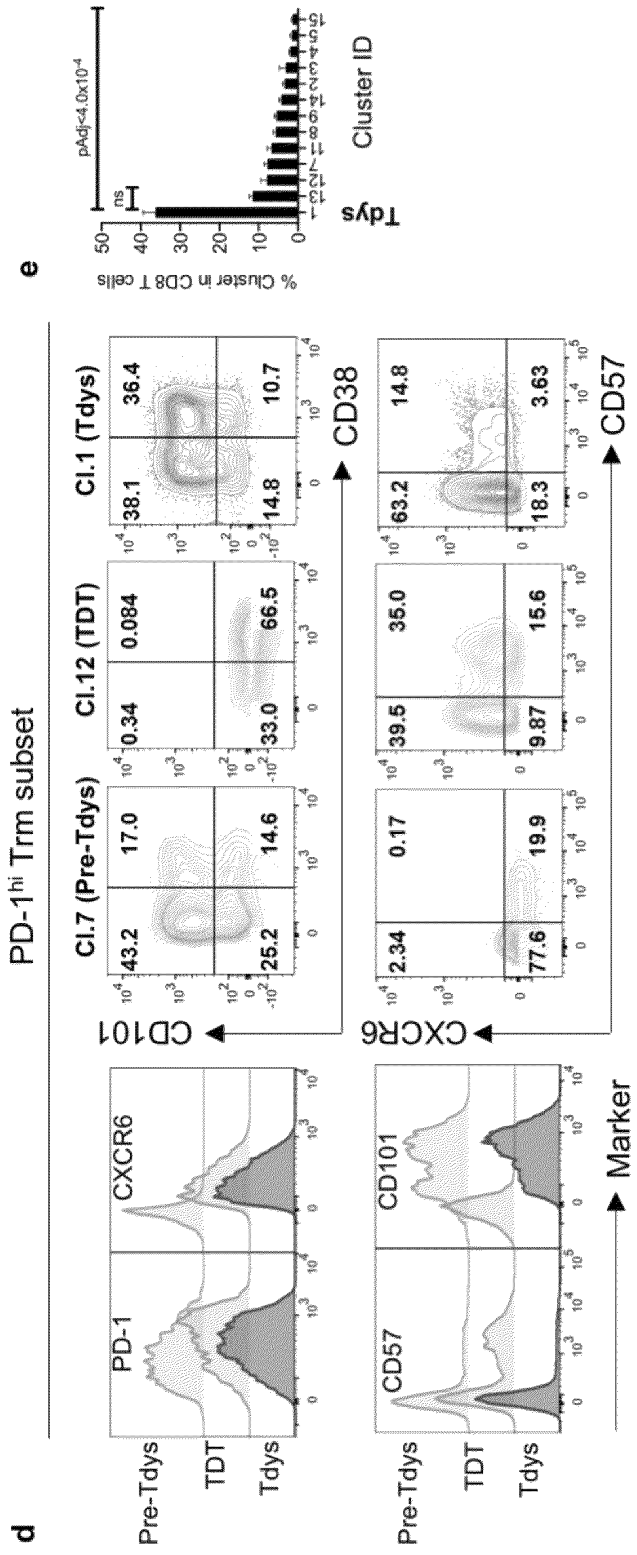


Figure 15 (continued)

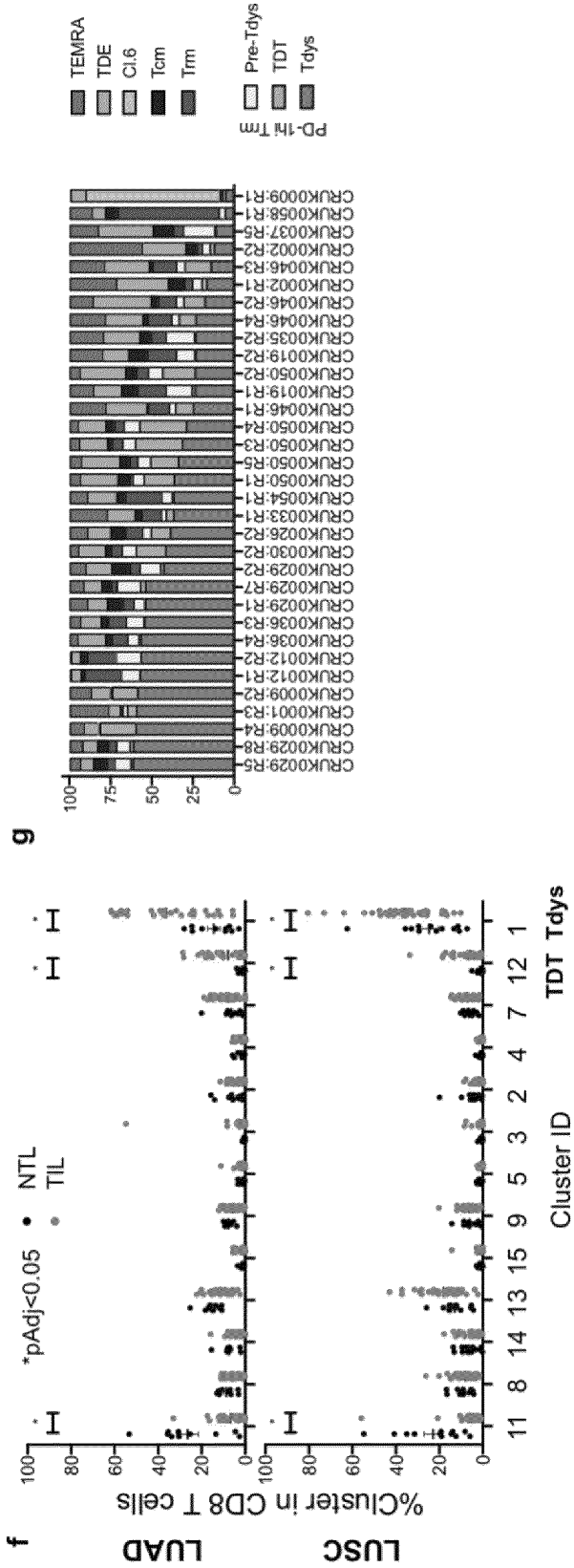


Figure 15 (continued)

Subset	Clusters	Key markers	Features	Relationship with tumor genomics
T_{EMRA}	8,11 (10, filtered, <1% freq)	CD45RA+ CD103- CD57+/-	Recirculating, cytotoxic, end stage memory cells, short telomeres	
TDE	13,14,15	CD45RA- CD103- CD57+	Recirculating cytotoxic, mature effectors	Decreased in presence of immune escape
Tcm	9	CD45RA- CD27+ CD28+ CD57- CD5hi	Recirculating Progenitor memory pool, low cytotoxicity, access to LN	Decreased with rising neoantigen burden
Trm	2,3,4,5	CD45RA- CD103+ CD27lo	Non recirculating, stably reside in non-lymphoid organs Low cytotoxicity/ classic effector function.	Decreased with rising neoantigen burden (cl.2,3,5)
PD-1^{hi} Trm	7,12,1	CD45RA- CD103+ PD1hi CD27hi FAShi CD57+/-	Highly proliferative, produce CXCL13, associated with improved response to anti-PD-1 in NSCLC, arise from chronic neoantigen stimulation in murine models, shown to harbor tumor reactive clones and populations lacking effector function.	
Pre-Tdys	7	CXCR6- CD57- CD38+ CD101+		
Terminally Differentiated Dysfunctional (TDT)	12	CXCR6+ CD57+ CD38hi CD101-		NS association with neoantigen burden
Dysfunctional (Tdys)	1	CXCR6+ CD57- CD38+ CD101+		Increased with neoantigen burden Increased in tumors with immune escape

Figure 16

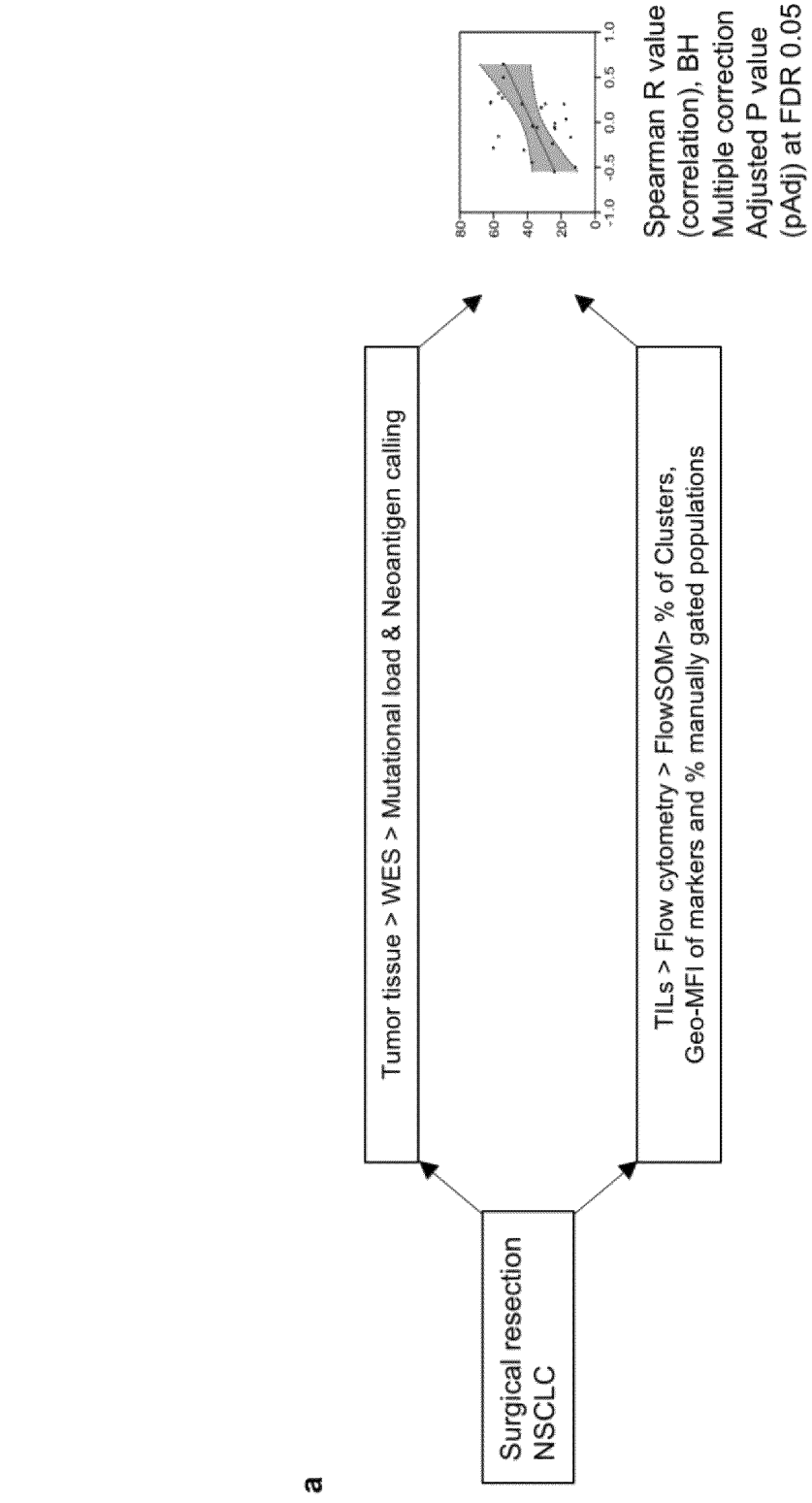

a
Surgical resection
NSCLC
Tumor tissue > WES > Mutational load & Neoantigen calling
TILs > Flow cytometry > FlowSOM> % of Clusters,
Geo-MFI of markers and % manually gated populations
Spearman R value
(correlation), BH
Multiple correction
Adjusted P value
(pAdj) at FDR 0.05
80
60
40
20
0
-1.0
-0.5
0.0
0.5
1.0

Figure 17

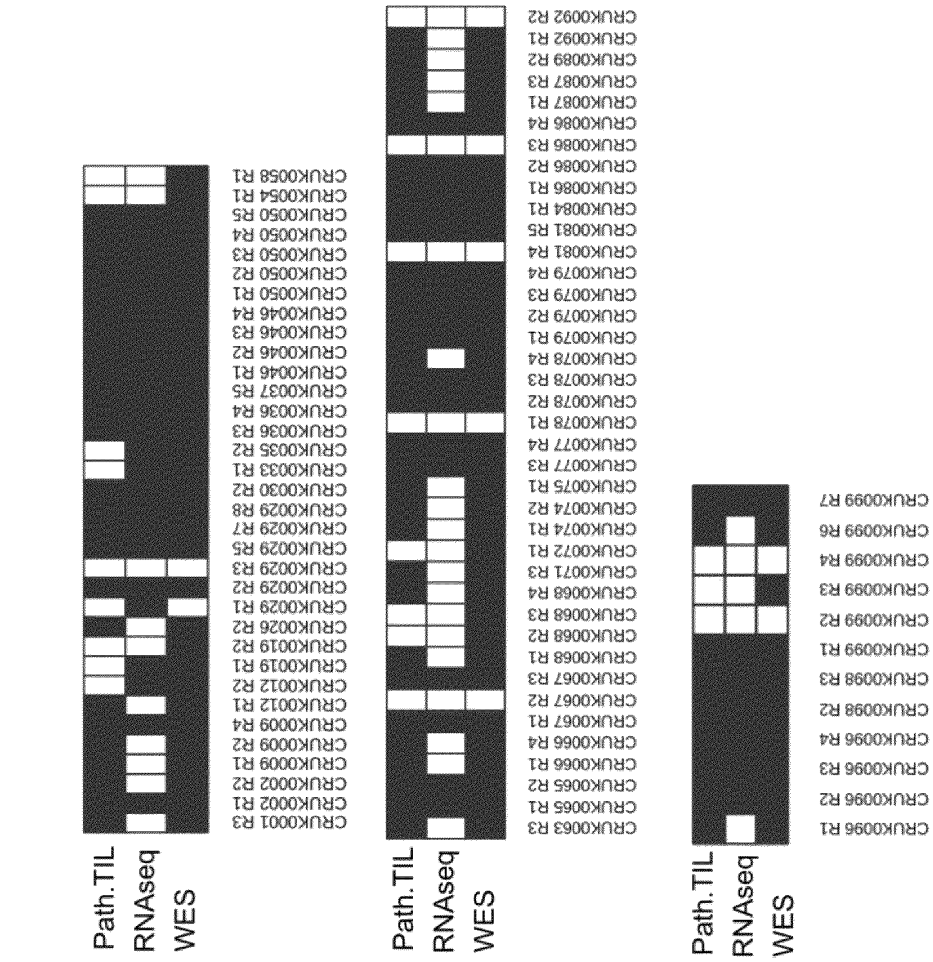


Figure 17 (continued)

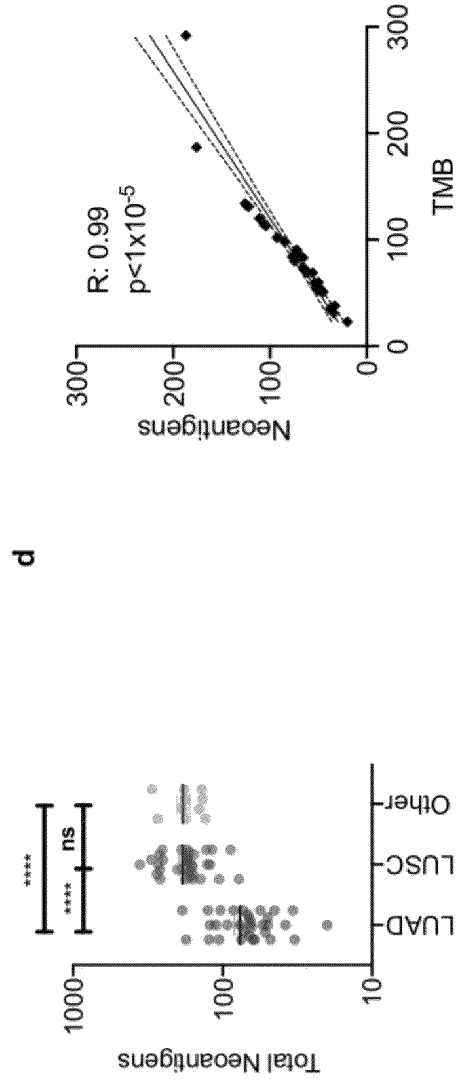


Figure 17 (continued)

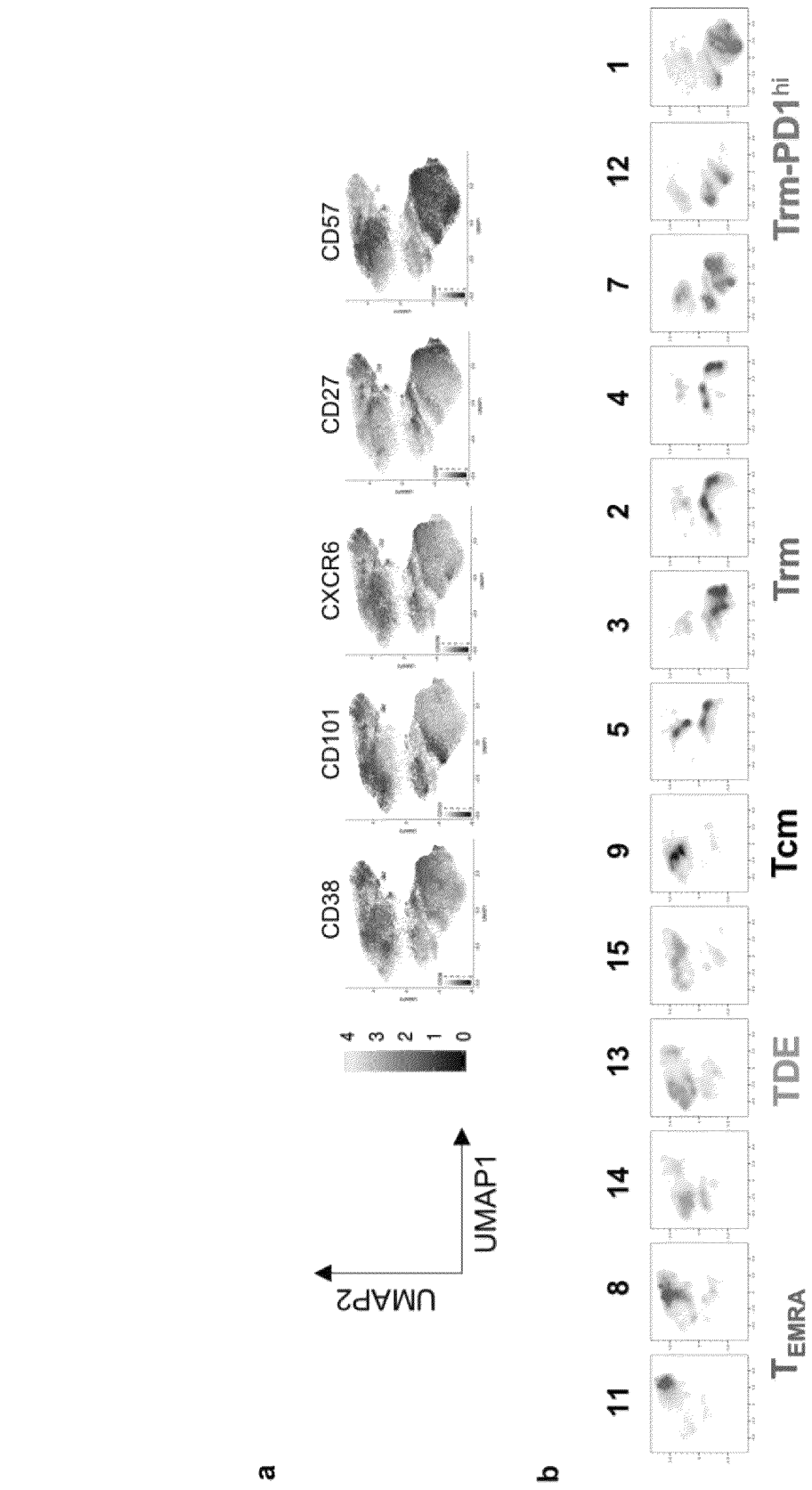

a
CD38
CD101
CXCR6
CD27
CD57
4
3
2
1
0
UMAP2
UMAP1
b
11
8
14
13
15
9
5
3
2
4
7
12
1
TEMRA
TDE
Tcm
Trm
Trm-PD1hi

Figure 18

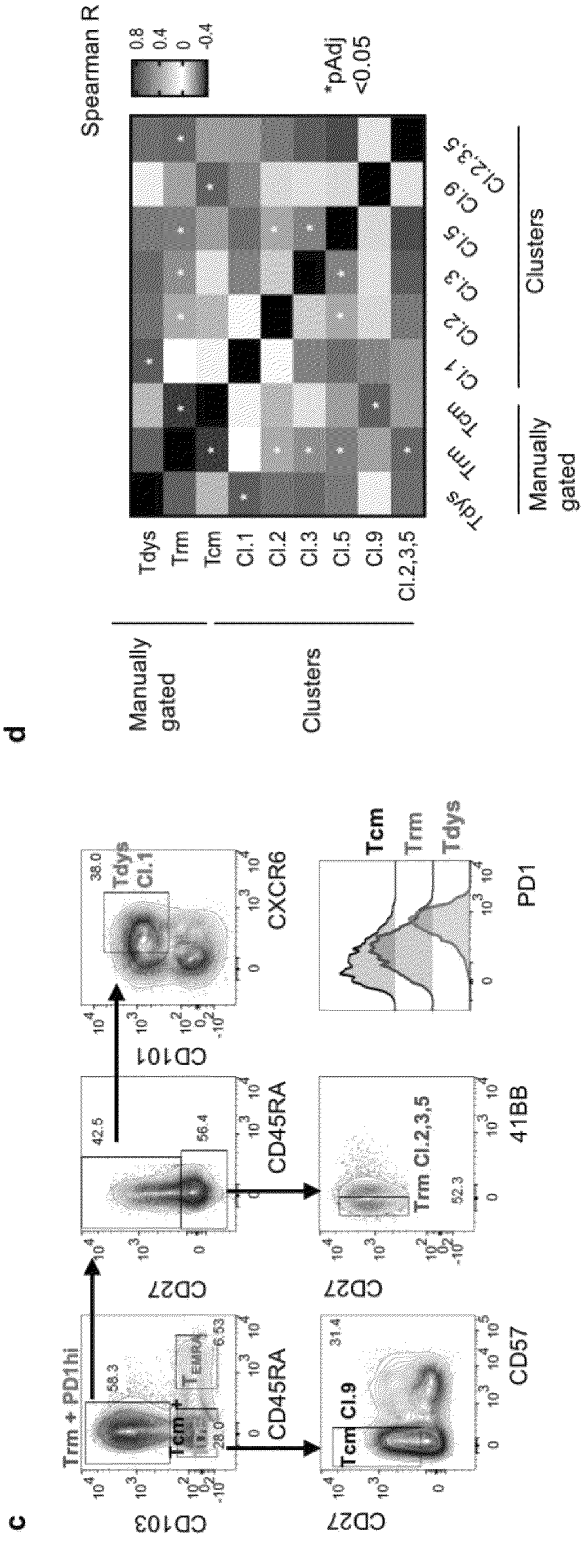


Figure 18 (continued)

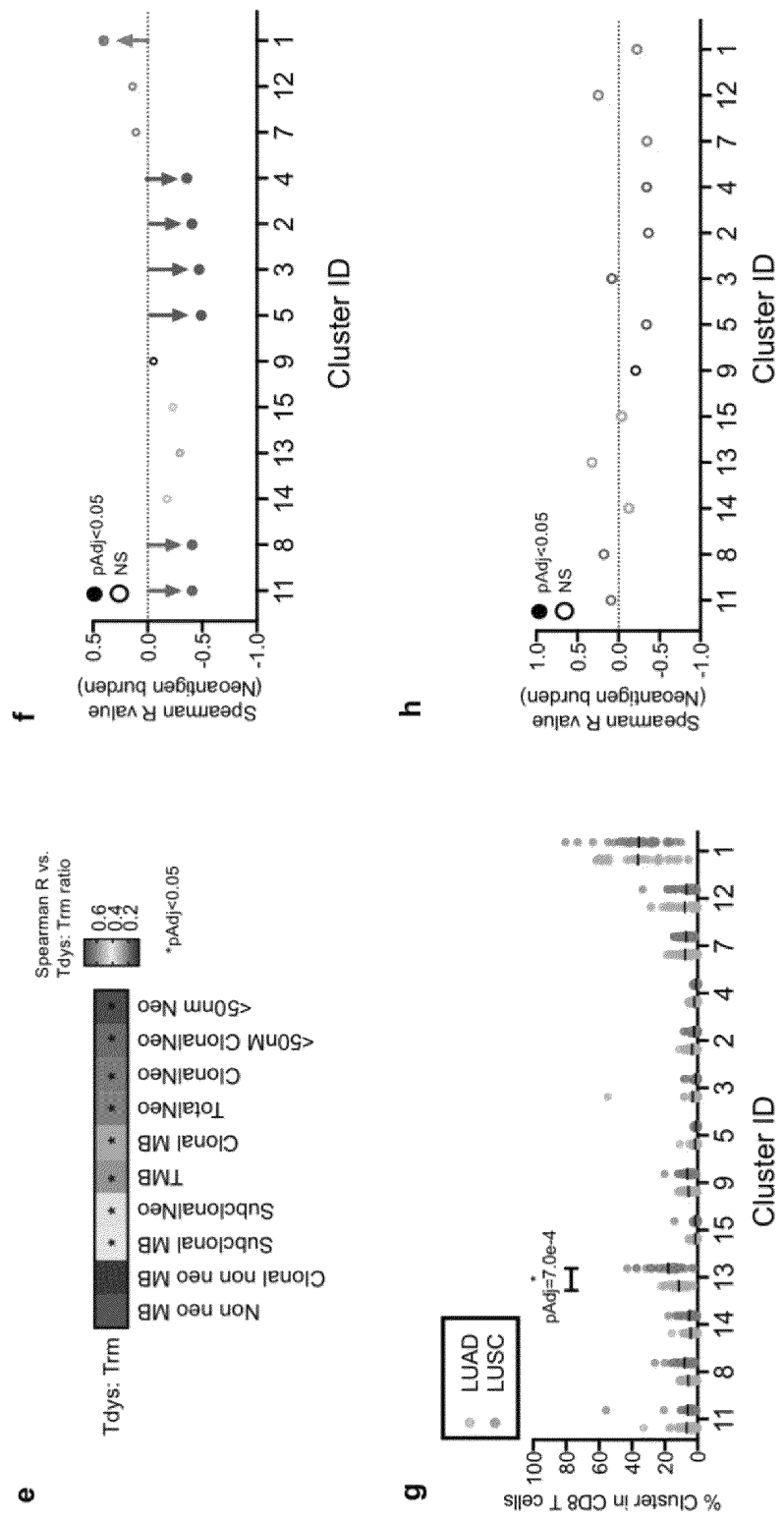


Figure 18 (continued)

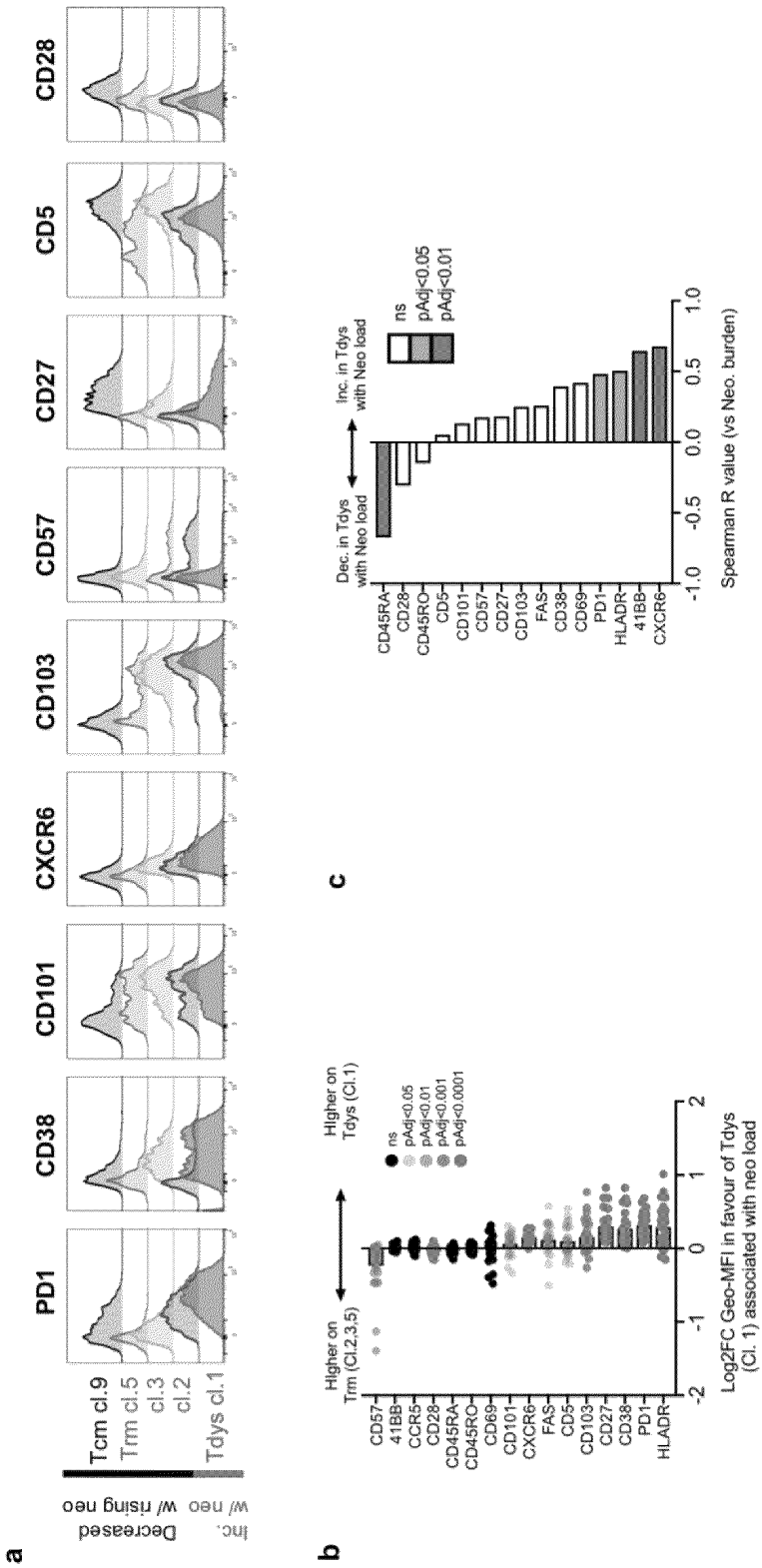

a
PD1
CD38
CD101
CXCR6
CD103
CD57
CD27
CD5
CD28
Tcm cl.9
Trm cl.5
cl.3
cl.2
Tdys cl.1
Decreased w/ rising neo
Inc. w/ neo
b
Higher on Trm (Cl.2.3.5)
Higher on Tdys (Cl.1)
ns
pAdj<0.05
pAdj<0.01
pAdj<0.001
pAdj<0.0001
CD57
41BB
CCR5
CD28
CD45RA
CD45RO
CD69
CD101
CXCR6
FAS
CD5
CD103
CD27
CD38
PD1
HLADR
-2
-1
0
1
2
Log2FC Geo-MFI in favour of Tdys (Cl. 1) associated with neo load
c
Dec. in Tdys with Neo load
Inc. in Tdys with Neo load
ns
pAdj<0.05
pAdj<0.01
CD45RA
CD28
CD45RO
CD5
CD101
CD57
CD27
CD103
FAS
CD38
CD69
PD1
HLADR
41BB
CXCR6
-1.0
-0.5
0.0
0.5
1.0
Spearman R value (vs Neo. burden)

Figure 19

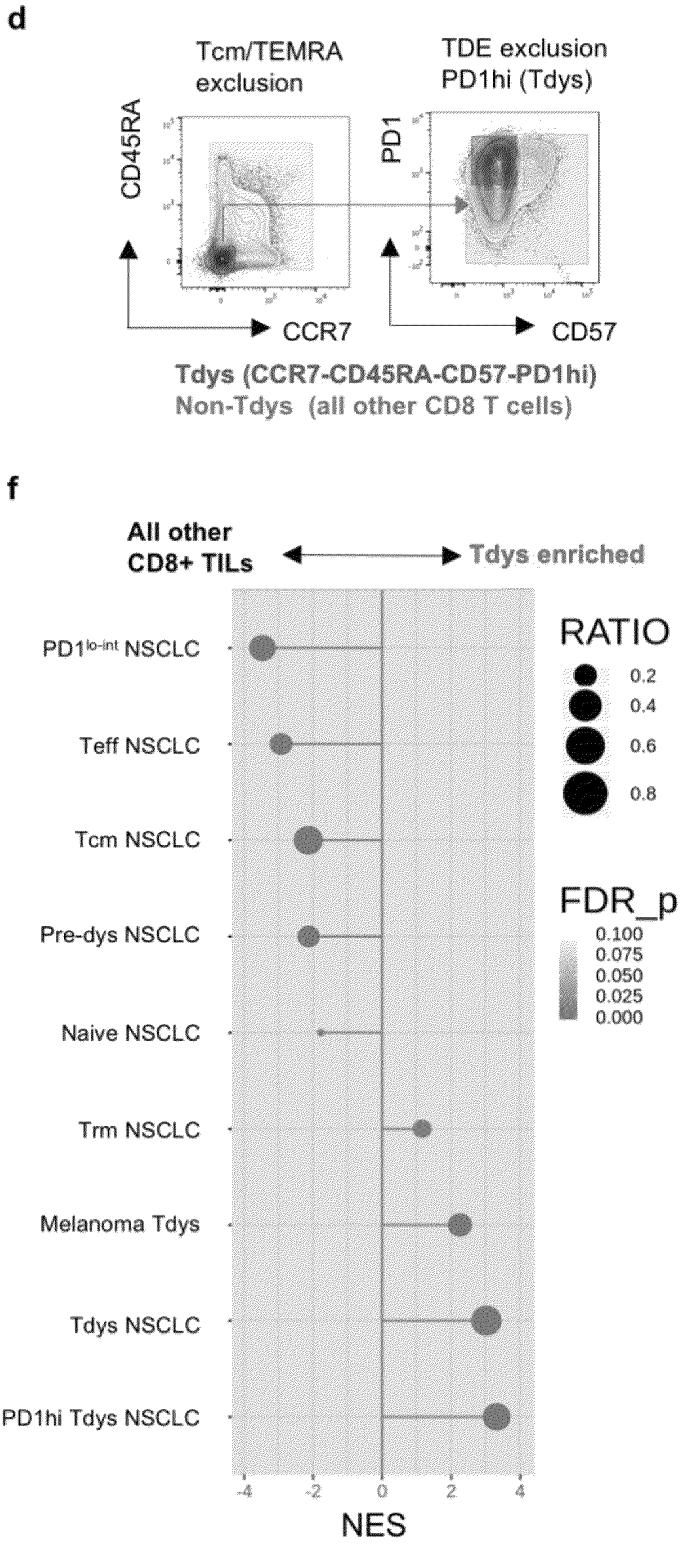
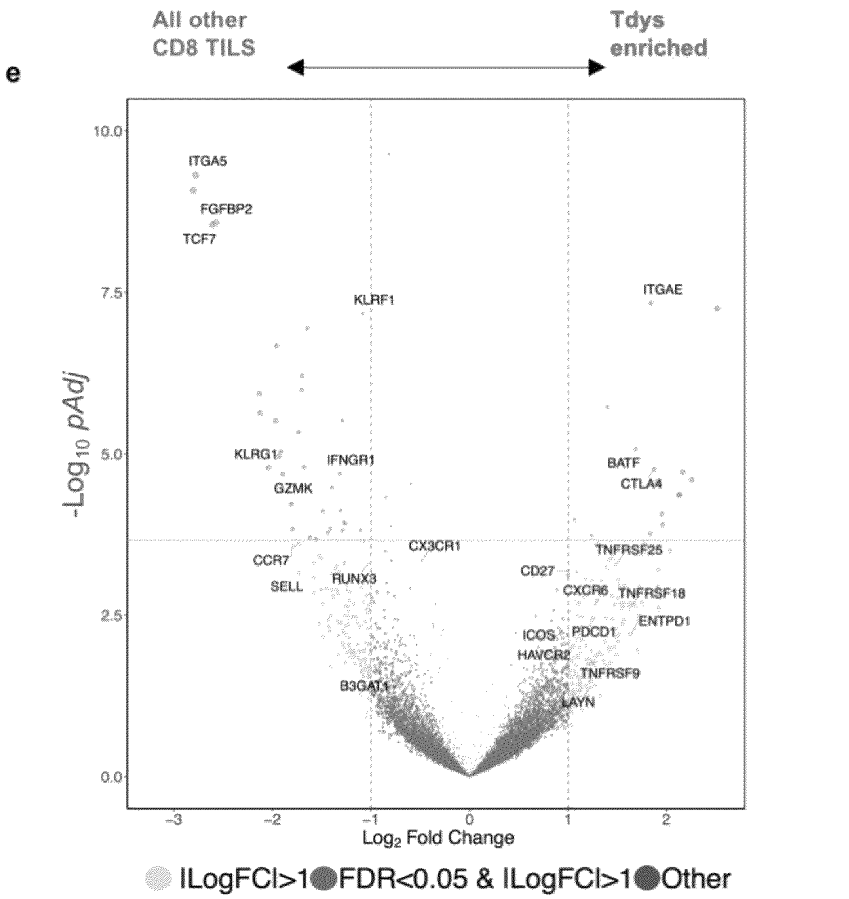


Figure 19 (continued)



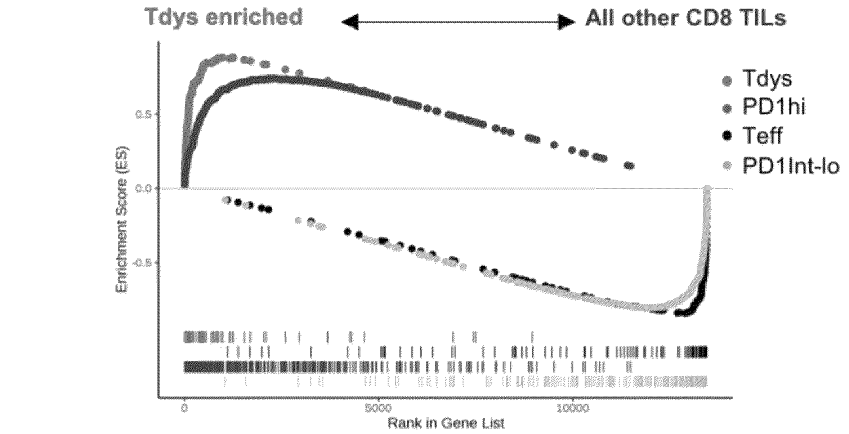


Figure 19 (continued)

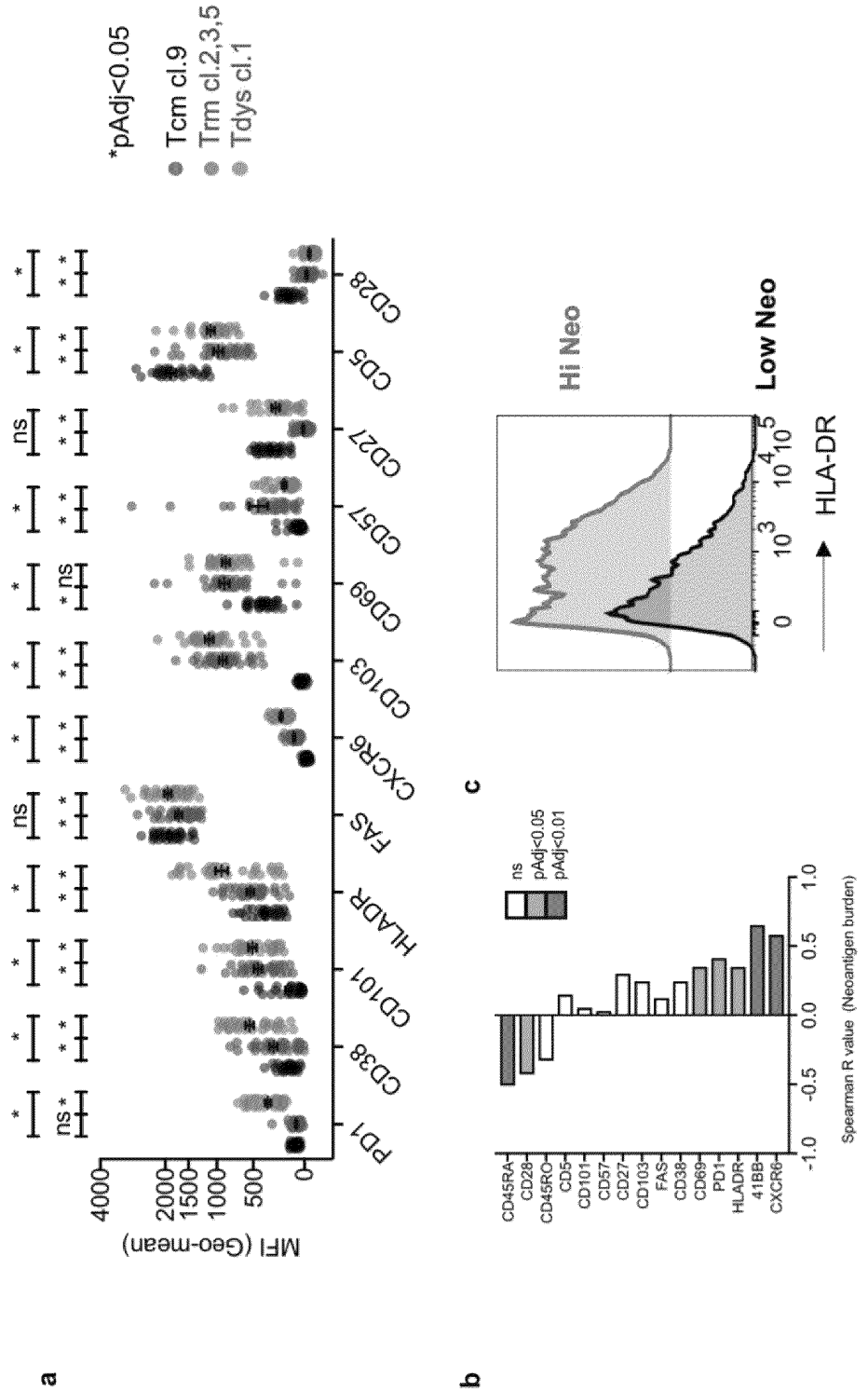
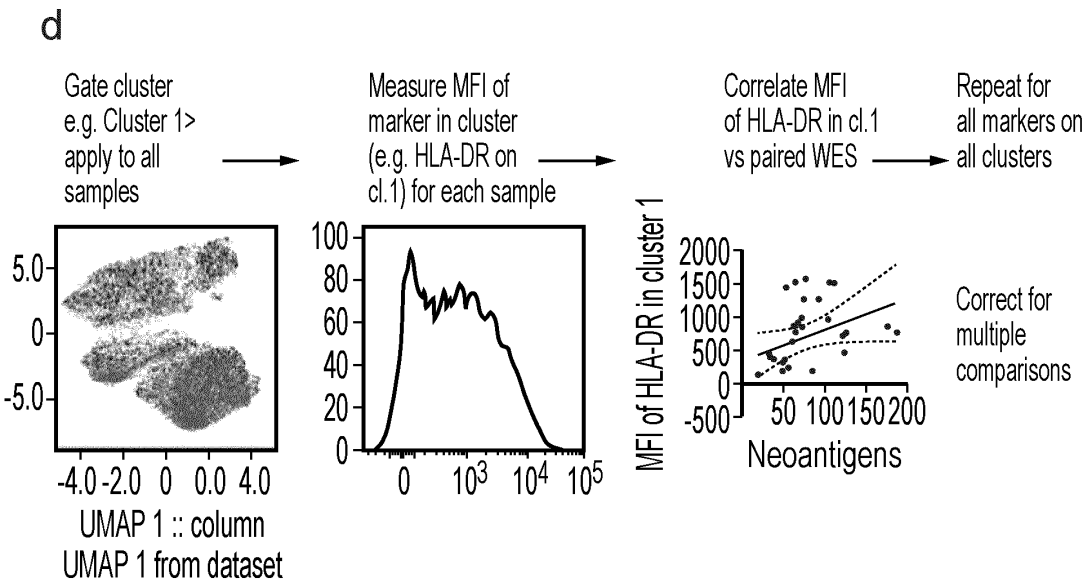

a
b
c
*pAdj<0.05
Tcm cl.9
Trm cl.2,3,5
Tdys cl.1
MFI (Geo-mean)
4000
2000
1500
1000
500
0
PD1
CD38
CD101
HLADR
FAS
CXCR6
CD103
CD69
CD57
CD27
CD5
CD28
ns
CD45RA
CD28
CD45RO
CD5
CD101
CD57
CD27
CD103
FAS
CD38
CD69
PD1
HLADR
41BB
CXCR6
pAdj<0.05
pAdj<0.01
-1.0
-0.5
0.0
0.5
1.0
Spearman R value (Neoantigen burden)
Hi Neo
Low Neo
HLA-DR

Figure 20



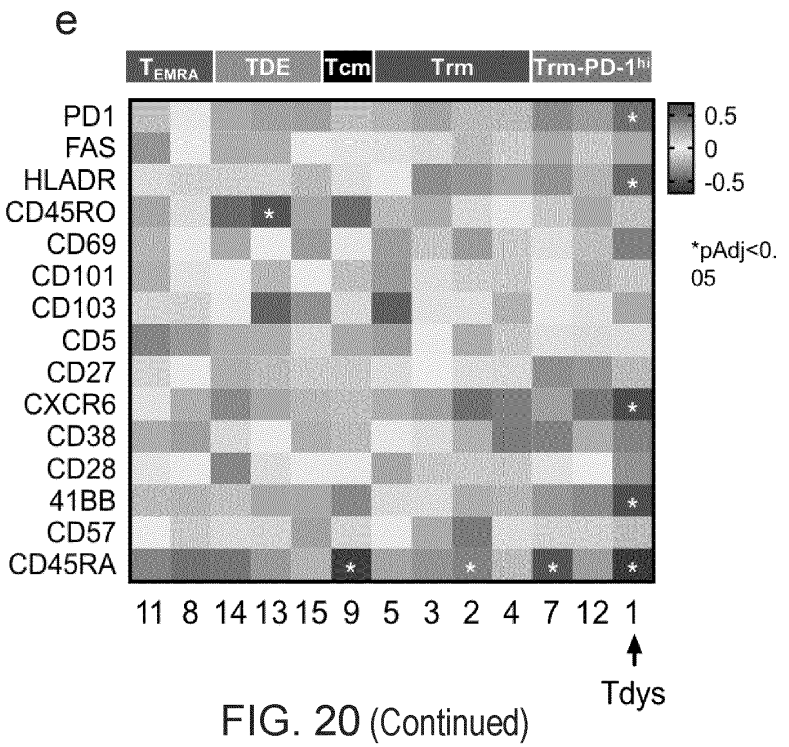


FIG. 20 (Continued)

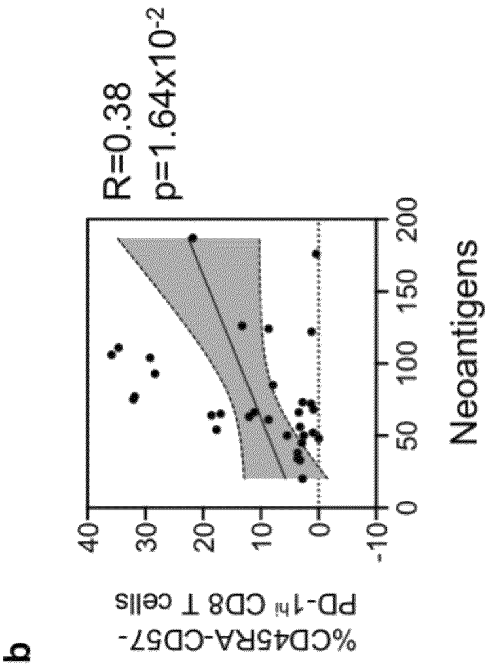

b
R=0.38
p=1.64x10-2
%CD45RA-CD57-
PD-1hi CD8 T cells
40
30
20
10
0
-10
0
50
100
150
200
Neoantigens
a
% CD8 T cells in cluster 1
80
60
40
20
0
p<1.0x10-5
****
Ungated
CD45RA-
CD57-
PD-1hi

Figure 21

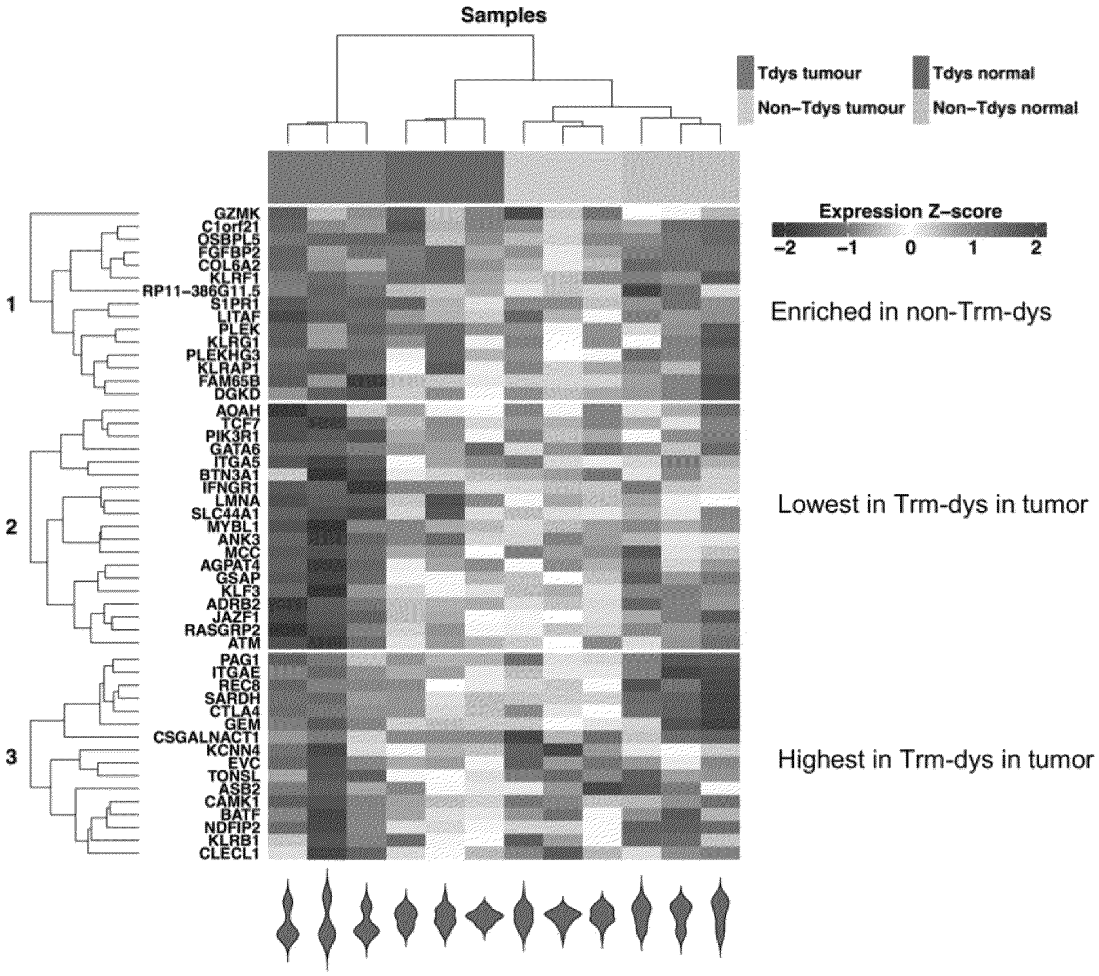

Samples
Tdys tumour
Tdys normal
Non−Tdys tumour
Non−Tdys normal
Expression Z−score
−2 −1 0 1 2
GZMK
C1orf21
OSBPL5
FGFBP2
COL6A2
KLRF1
RP11−386G11.5
S1PR1
LITAF
PLEK
KLRG1
PLEKHG3
KLRAP1
FAM65B
DGKD
AOAH
TCF7
PIK3R1
GATA6
ITGA5
BTN3A1
IFNGR1
LMNA
SLC44A1
MYBL1
ANK3
MCC
AGPAT4
GSAP
KLF3
ADRB2
JAZF1
RASGRP2
ATM
PAG1
ITGAE
REC8
SARDH
CTLA4
GEM
CSGALNACT1
KCNN4
EVC
TONSL
ASB2
CAMK1
BATF
NDFIP2
KLRB1
CLECL1
1
2
3
Enriched in non-Trm-dys
Lowest in Trm-dys in tumor
Highest in Trm-dys in tumor

Figure 21C

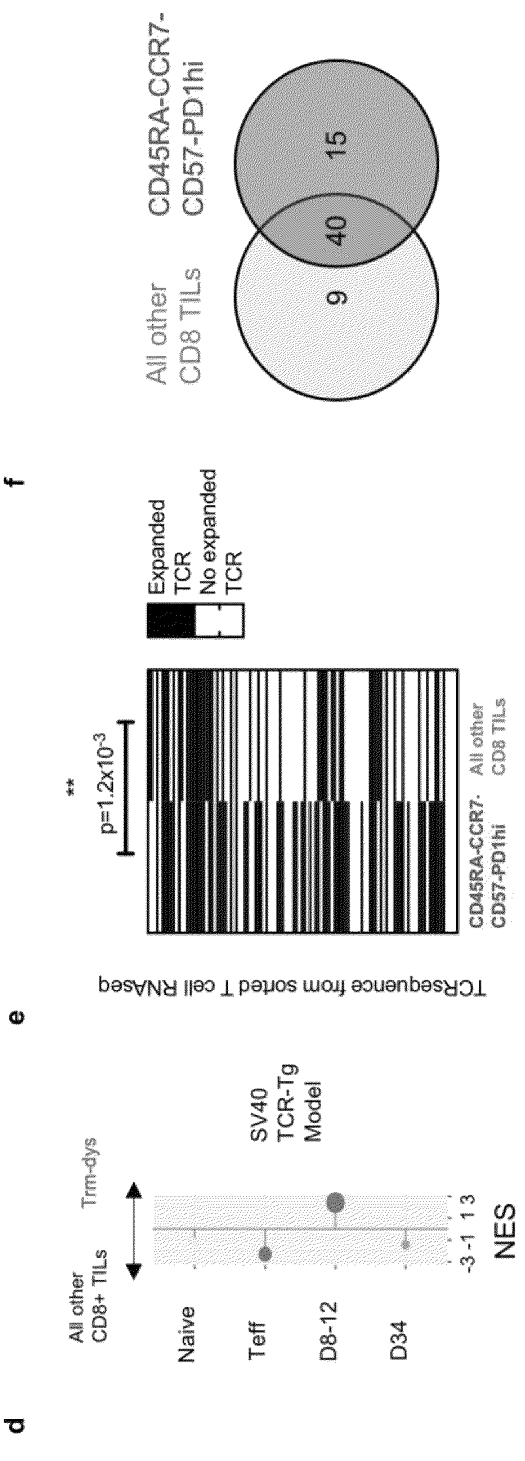
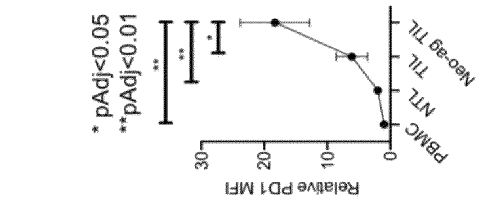
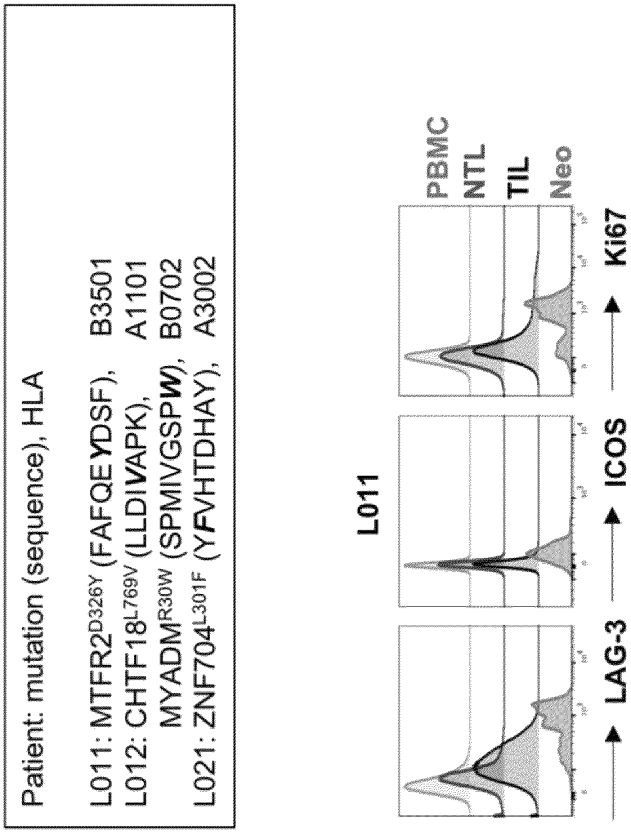


Figure 21 (continued)



b



a

c

Figure 22

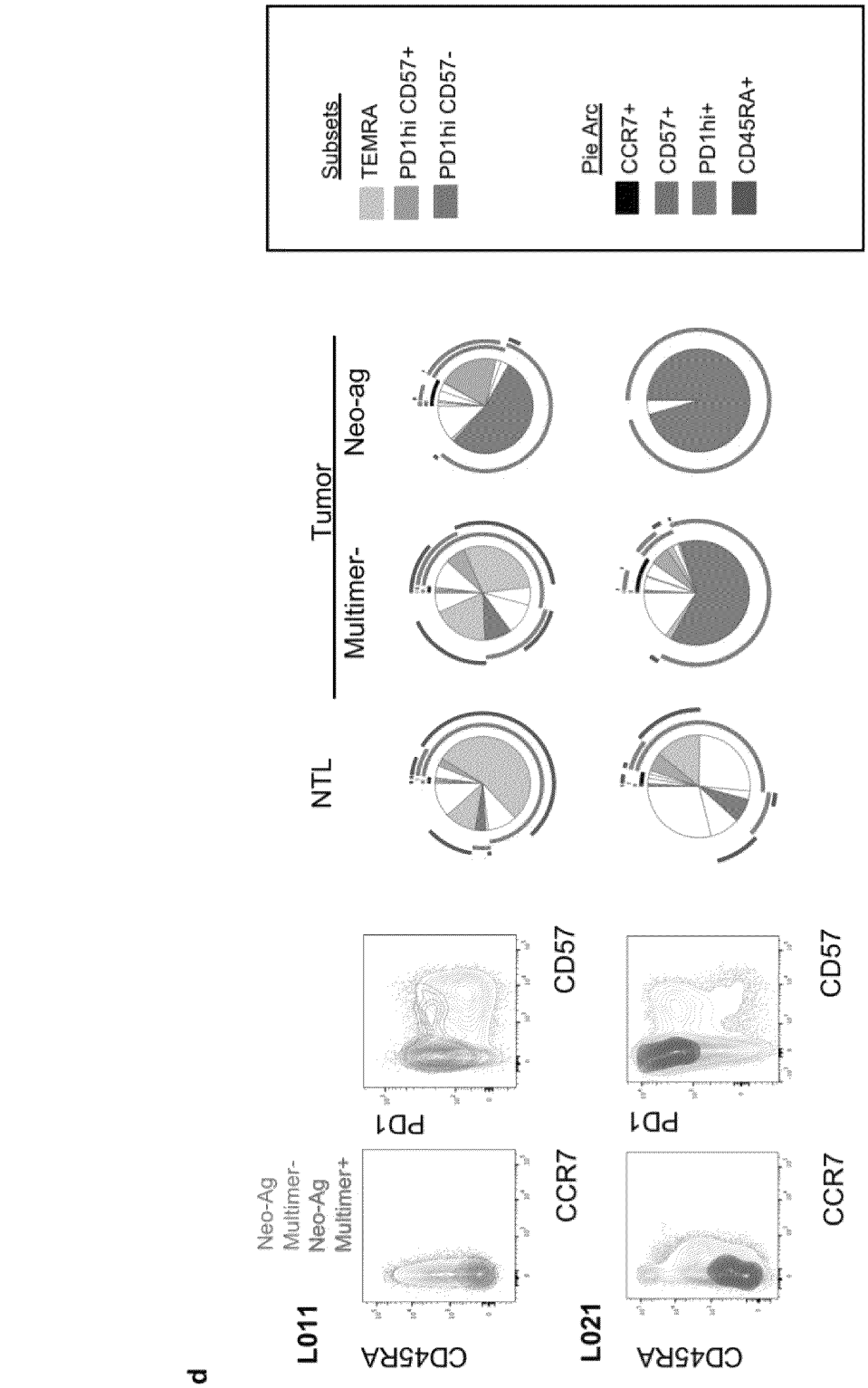
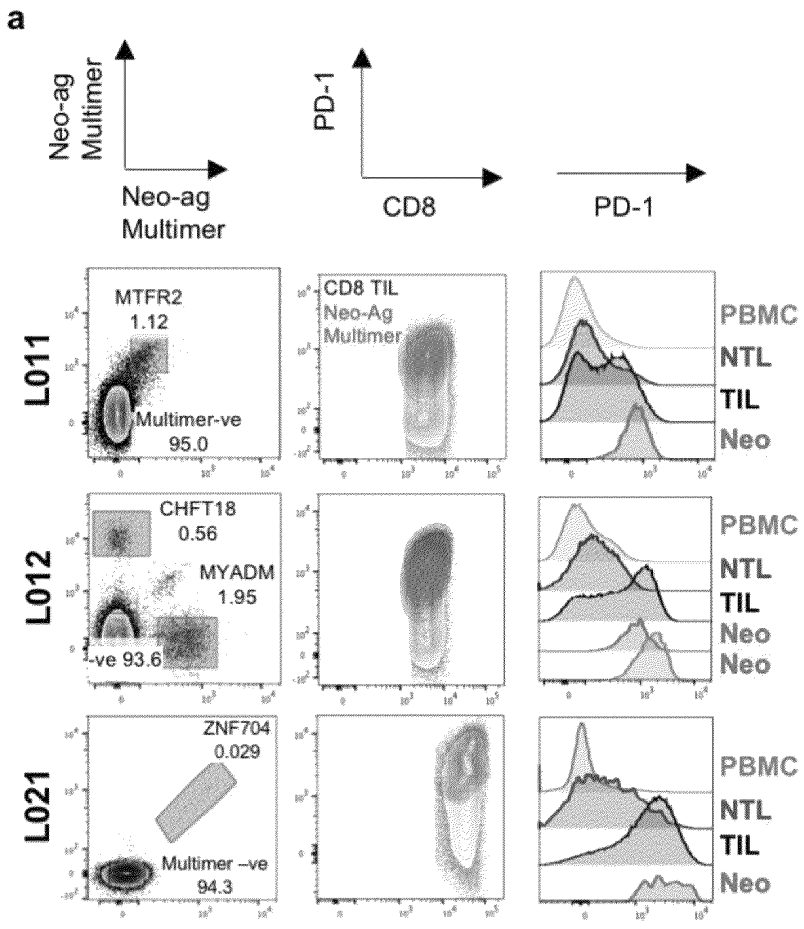


Figure 22 (continued)


a
Neo-ag Multimer
Neo-ag Multimer
PD-1
CD8
PD-1
L011
MTFR2
1.12
Multimer-ve
95.0
CD8 TIL
Neo-Ag
Multimer
PBMC
NTL
TIL
Neo
L012
CHFT18
0.56
MYADM
1.95
-ve 93.6
PBMC
NTL
TIL
Neo
Neo
L021
ZNF704
0.029
Multimer –ve
94.3
PBMC
NTL
TIL
Neo

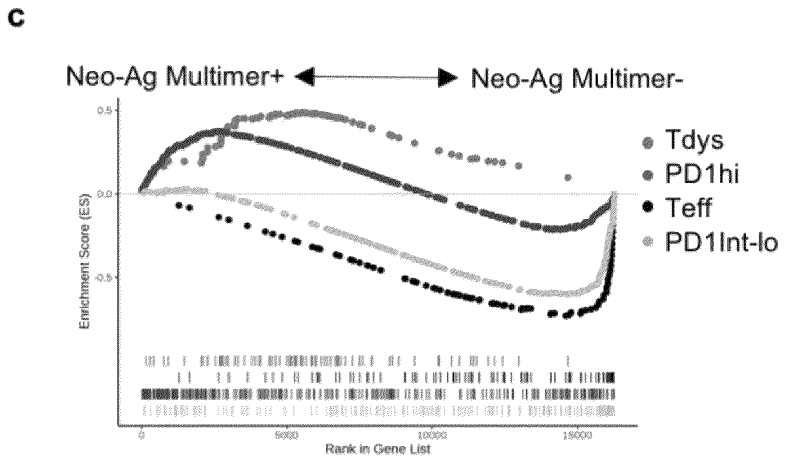

c
Neo-Ag Multimer+
Neo-Ag Multimer-
Enrichment Score (ES)
0.5
0.0
-0.5
0
5000
10000
15000
Rank in Gene List
Tdys
PD1hi
Teff
PD1Int-lo

Figure 23

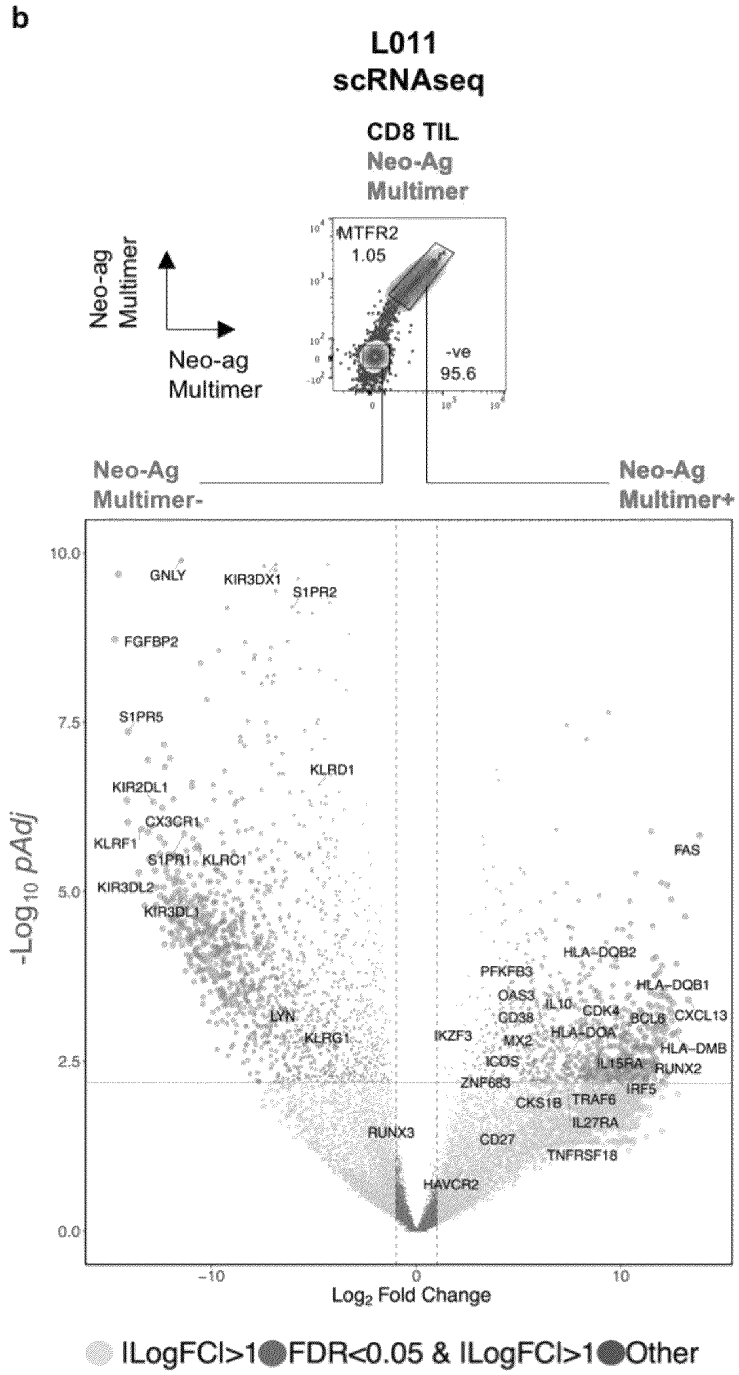


Figure 23 (continued)

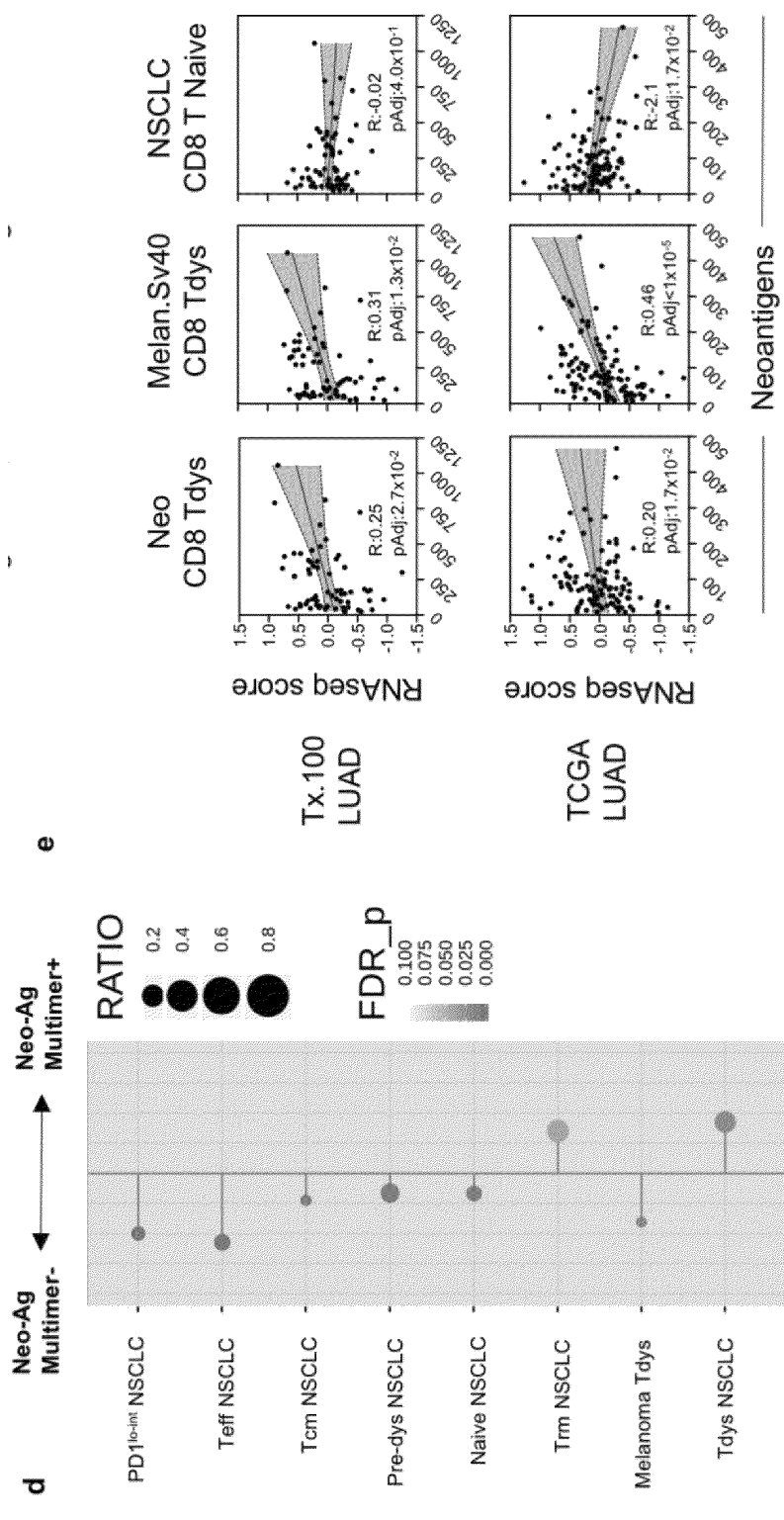


Figure 23 (continued)

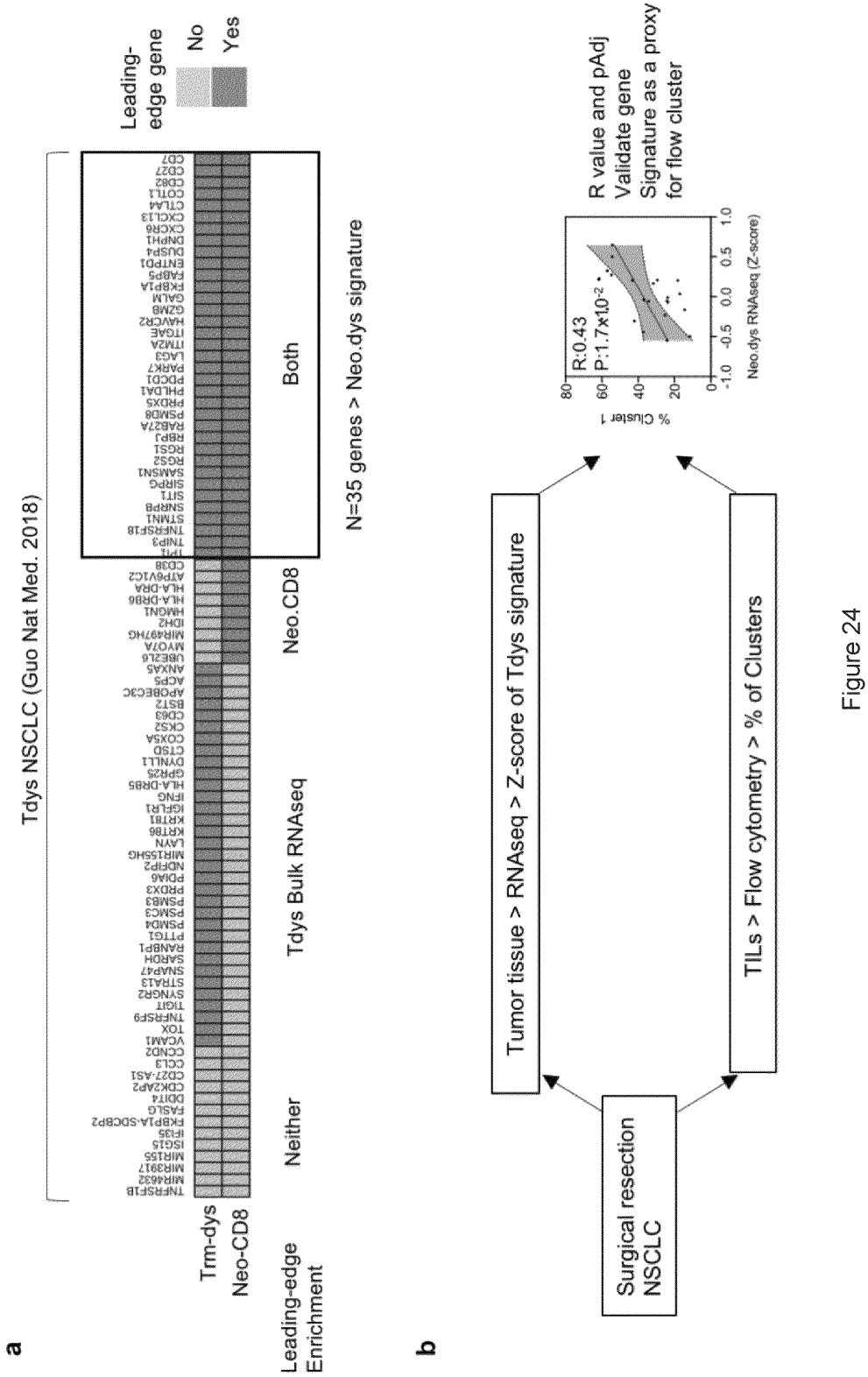

a
Tdys NSCLC (Guo Nat Med. 2018)
Leading-edge gene
No
Yes
Trm-dys
Neo-CD8
Leading-edge Enrichment
Neither
Tdys Bulk RNAseq
Neo.CD8
Both
N=35 genes > Neo.dys signature
b
Surgical resection NSCLC
Tumor tissue > RNAseq > Z-score of Tdys signature
TILs > Flow cytometry > % of Clusters
R value and pAdj
Validate gene
Signature as a proxy
for flow cluster
R:0.43
P:1.7x10-2
% Cluster 1
Neo.dys RNAseq (Z-score)

Figure 24

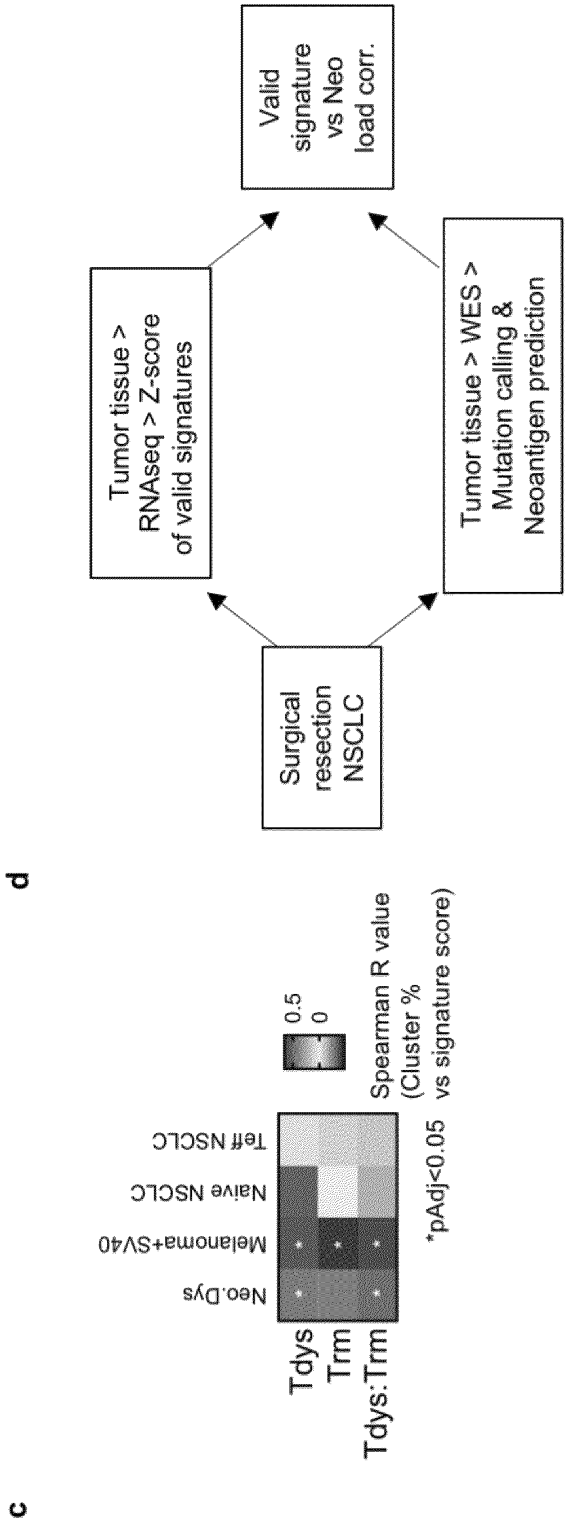


Figure 24 (continued)

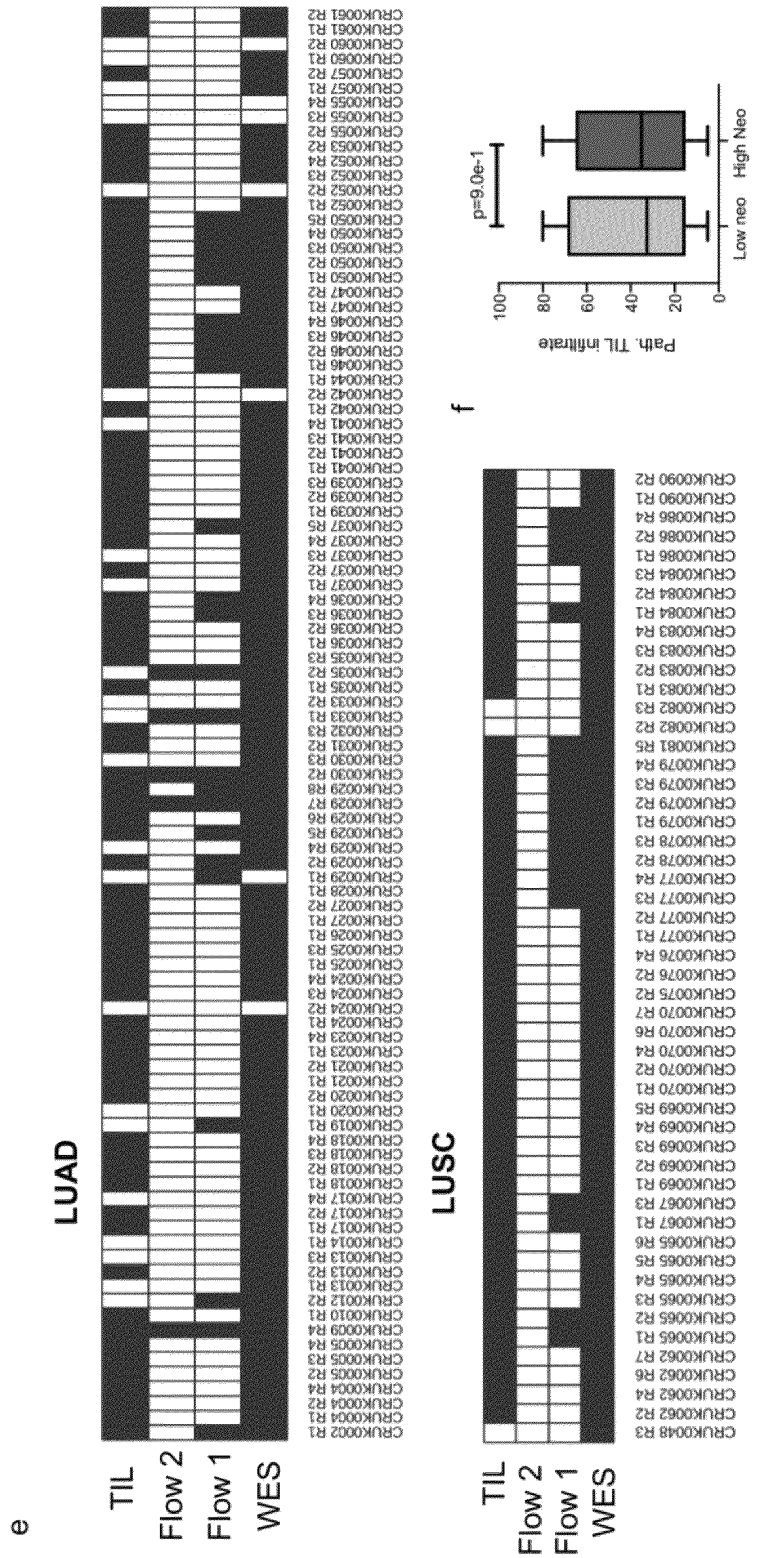


Figure 24 (continued)

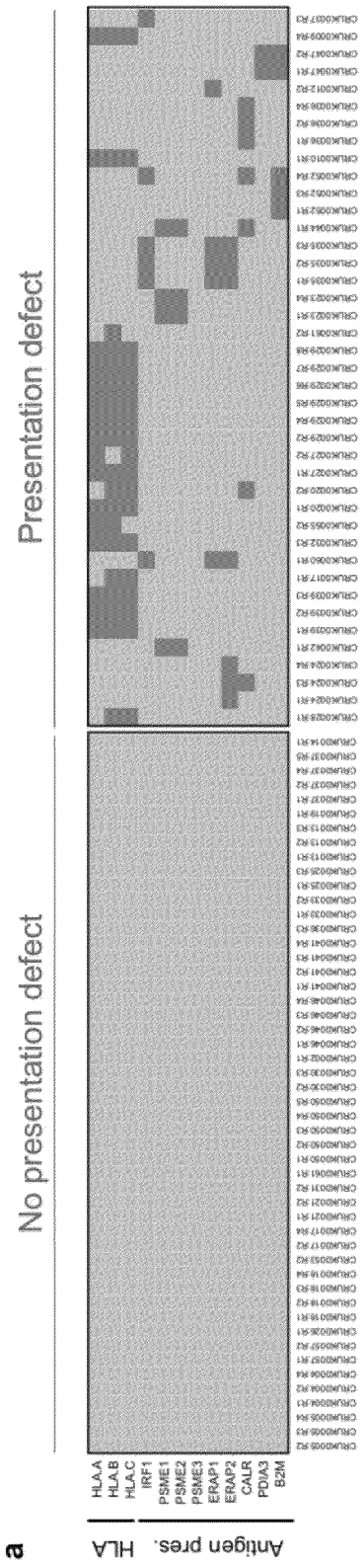

a
HLA
Antigen pres.
HLA.A
HLA.B
HLA.C
IRF1
PSME1
PSME2
PSME3
ERAP1
ERAP2
CALR
PDIA3
B2M
No presentation defect
Presentation defect

Figure 25

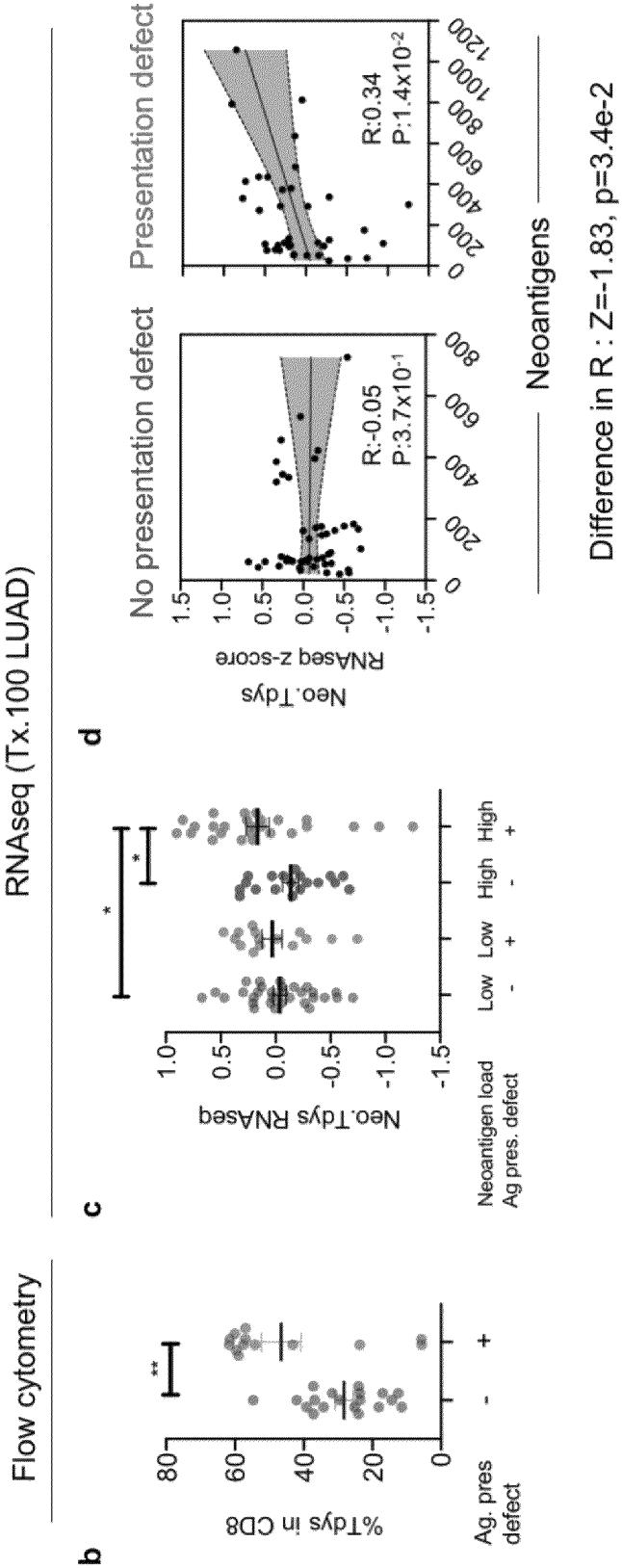


Figure 25 (continued)

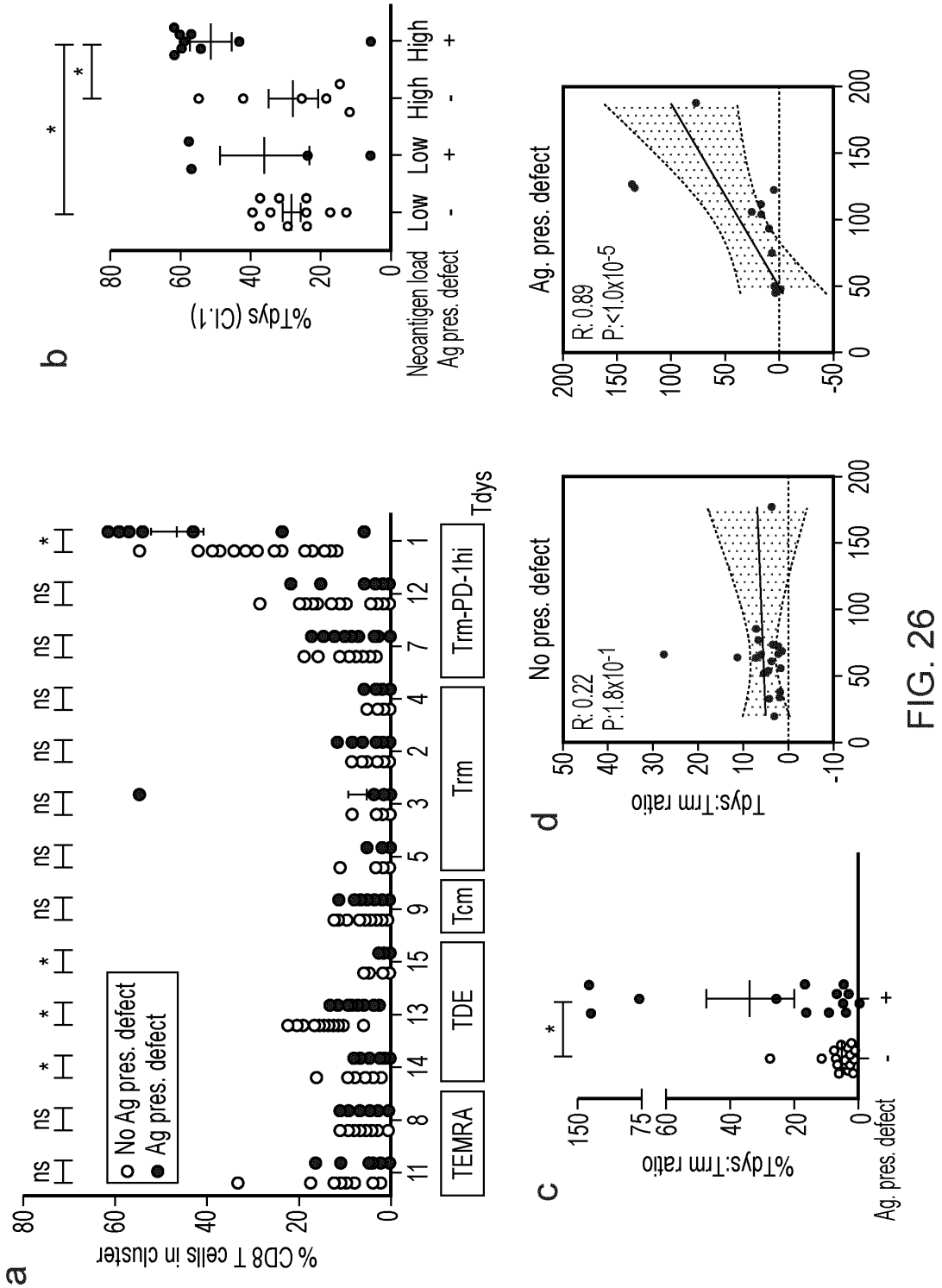

a
% CD8 T cells in cluster
No Ag pres. defect
Ag pres. defect
TEMRA
TDE
Tcm
Trm
Trm-PD-1hi
Tdys
b
%Tdys (Cl.1)
Neoantigen load
Ag pres. defect
c
%Tdys:Trm ratio
Ag. pres. defect
d
No pres. defect
R: 0.22
P:1.8x10-1
Ag. pres. defect
R: 0.89
P:<1.0x10-5
Tdys:Trm ratio

FIG. 26

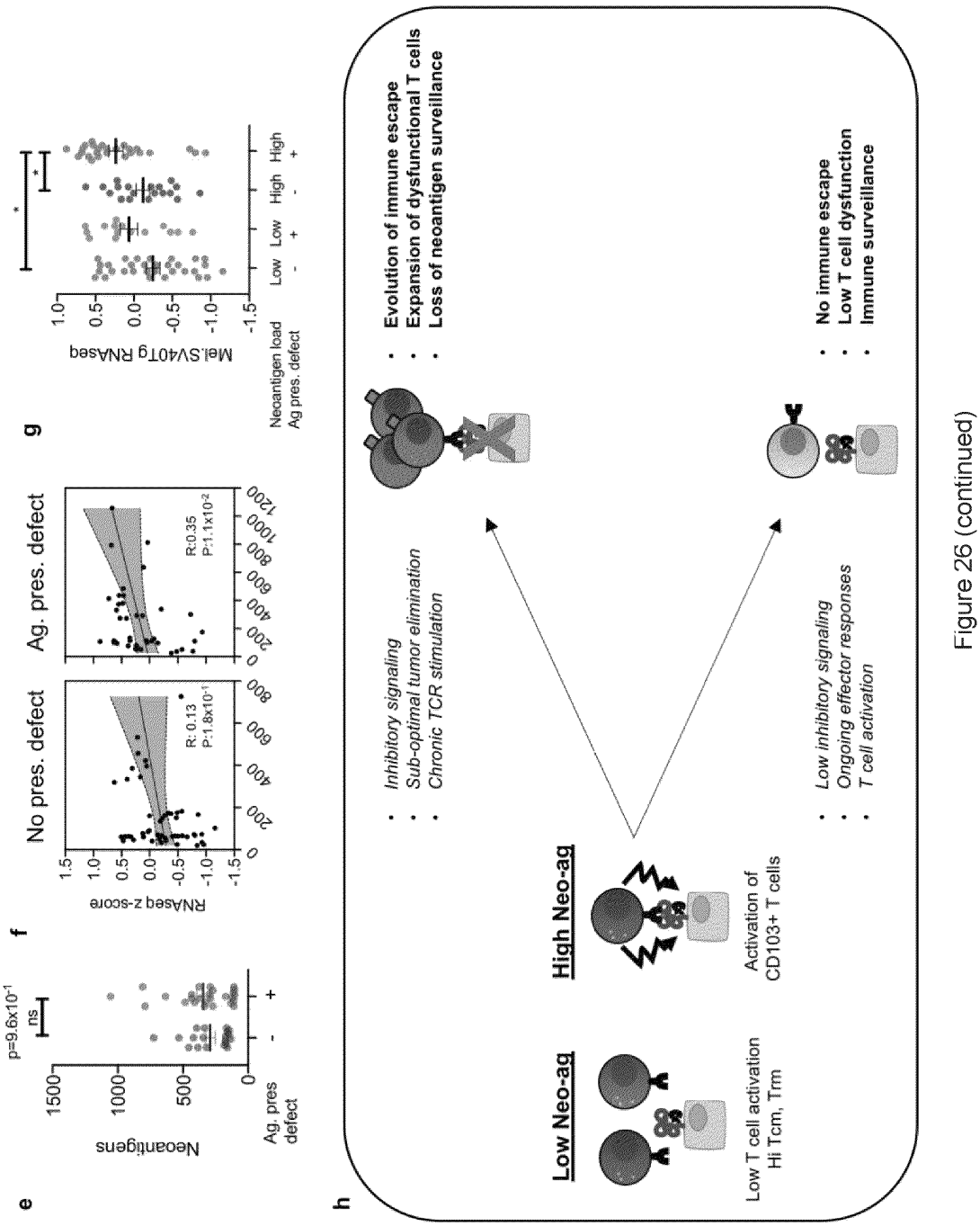


Figure 26 (continued)

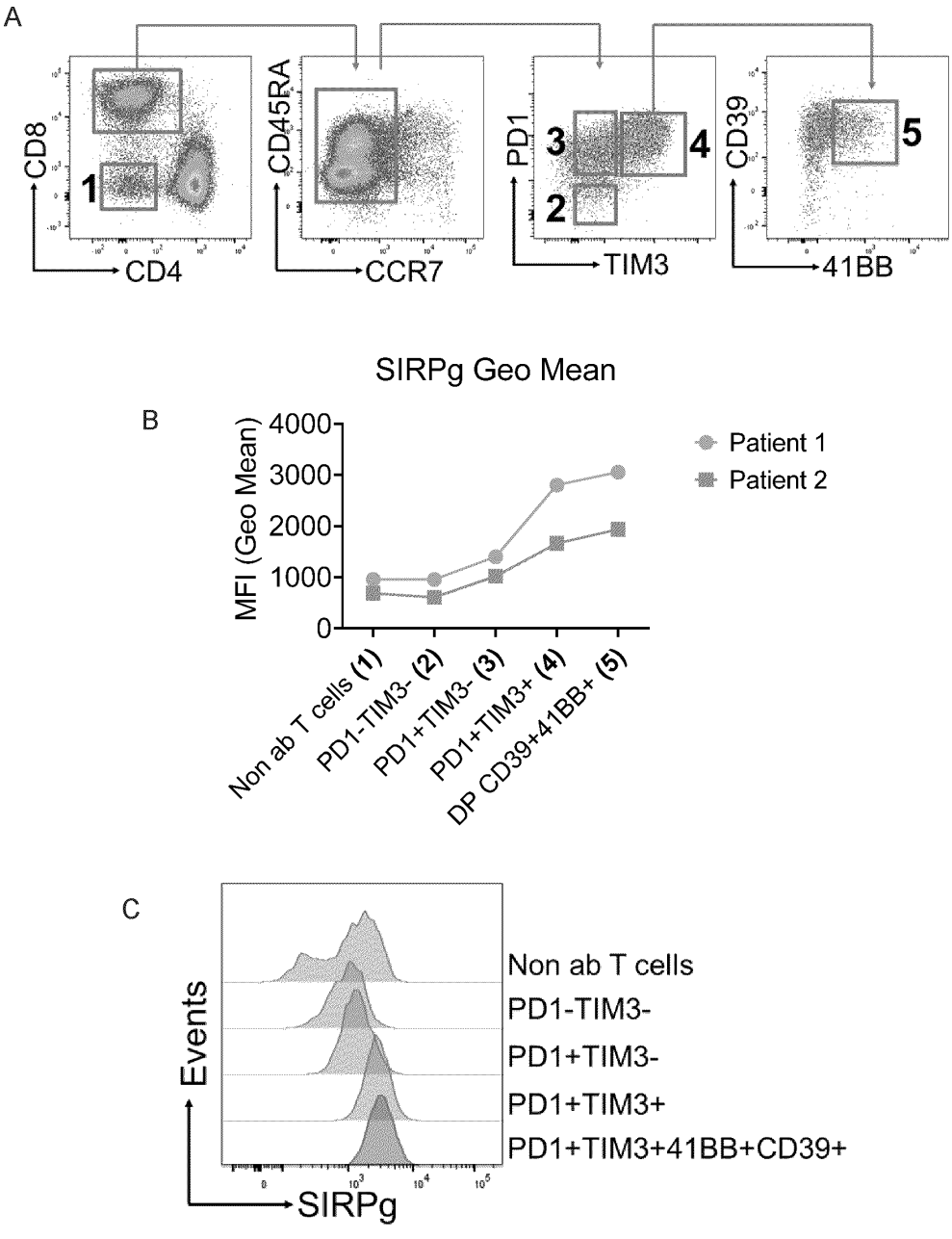
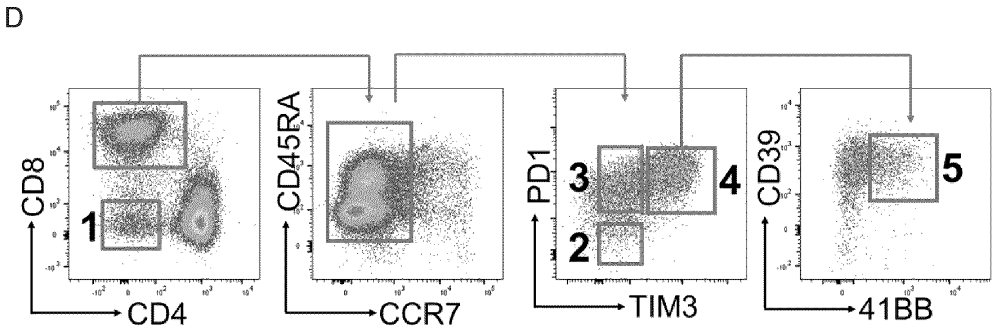
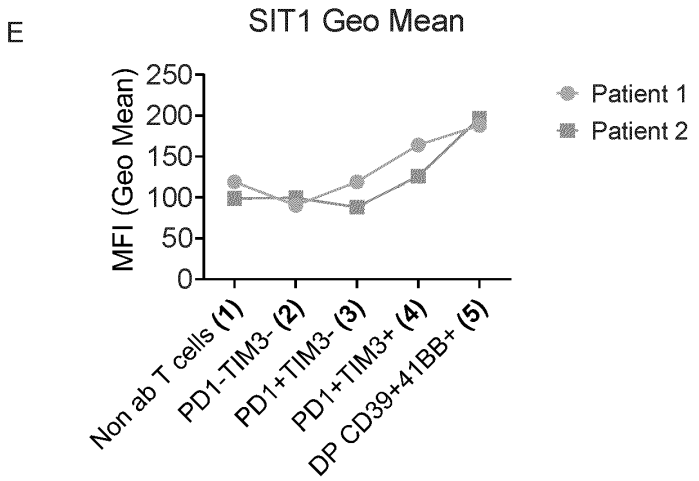

A
CD8
CD4
1
CD45RA
CCR7
PD1
TIM3
2
3
4
CD39
41BB
5
B
SIRPg Geo Mean
MFI (Geo Mean)
4000
3000
2000
1000
0
Patient 1
Patient 2
Non ab T cells (1)
PD1-TIM3- (2)
PD1+TIM3- (3)
PD1+TIM3+ (4)
DP CD39+41BB+ (5)
C
Events
SIRPg
Non ab T cells
PD1-TIM3-
PD1+TIM3-
PD1+TIM3+
PD1+TIM3+41BB+CD39+

Figure 27





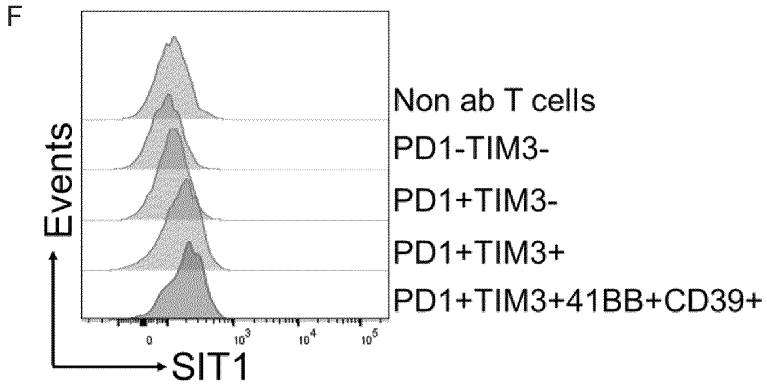
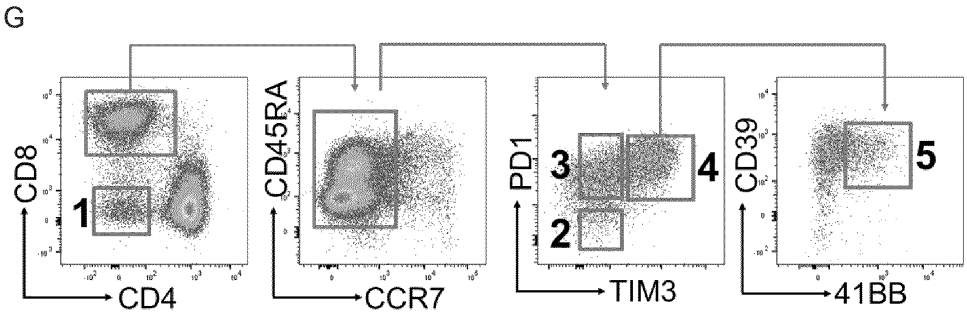
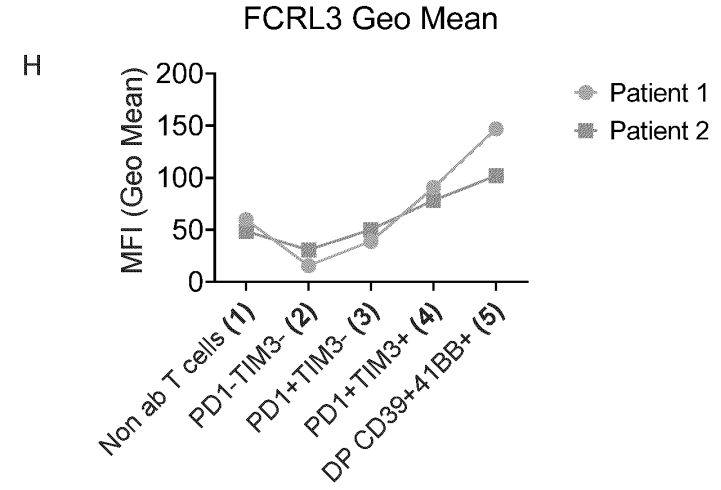


Figure 27 (continued)





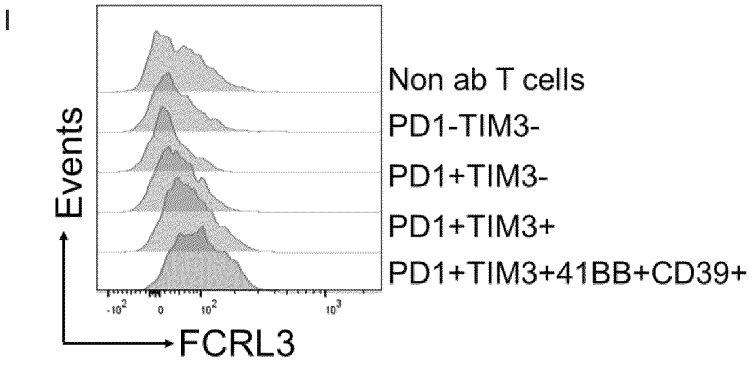


Figure 27 (continued)

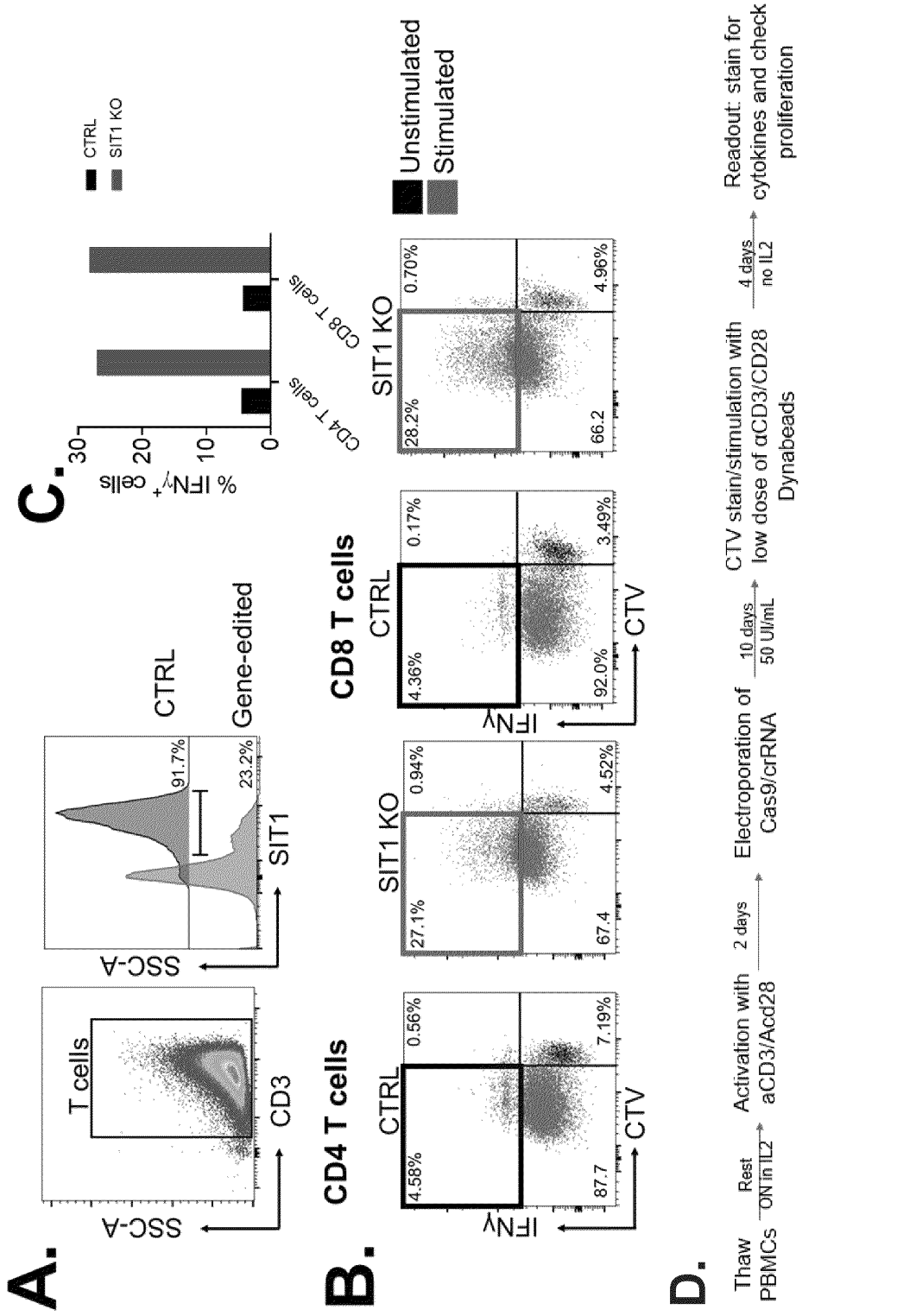

A.
T cells
SSC-A
CD3
CTRL
Gene-edited
91.7%
23.2%
SSC-A
SIT1
B.
CD4 T cells
CD8 T cells
CTRL
SIT1 KO
4.58%
0.56%
87.7
7.19%
27.1%
0.94%
67.4
4.52%
4.36%
0.17%
92.0%
3.49%
28.2%
0.70%
66.2
4.96%
IFNγ
CTV
C.
% IFNγ+ cells
30
20
10
0
CD4 T cells
CD8 T cells
CTRL
SIT1 KO
Unstimulated
Stimulated
D.
Thaw PBMCs
Rest ON in IL2
Activation with aCD3/Acd28
2 days
Electroporation of Cas9/crRNA
10 days 50 UI/mL
CTV stain/stimulation with low dose of αCD3/CD28 Dynabeads
4 days no IL2
Readout: stain for cytokines and check proliferation

Figure 28

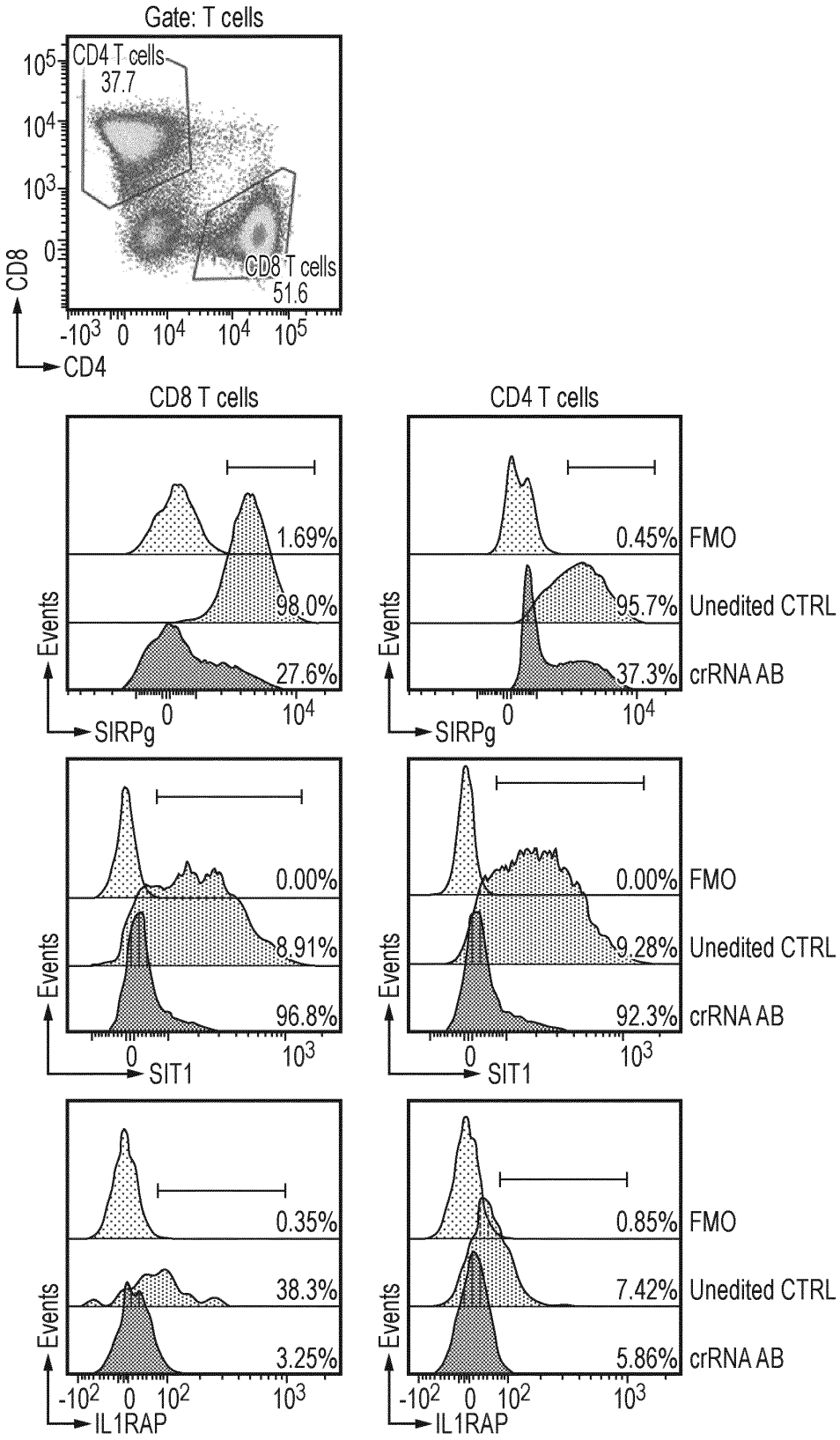

Gate: T cells
CD4 T cells
37.7
CD8 T cells
51.6
CD8
CD4
CD8 T cells
CD4 T cells
1.69%
98.0%
27.6%
0.45%
95.7%
37.3%
FMO
Unedited CTRL
crRNA AB
Events
SIRPg
0.00%
8.91%
96.8%
0.00%
9.28%
92.3%
SIT1
0.35%
38.3%
3.25%
0.85%
7.42%
5.86%
IL1RAP

FIG. 29

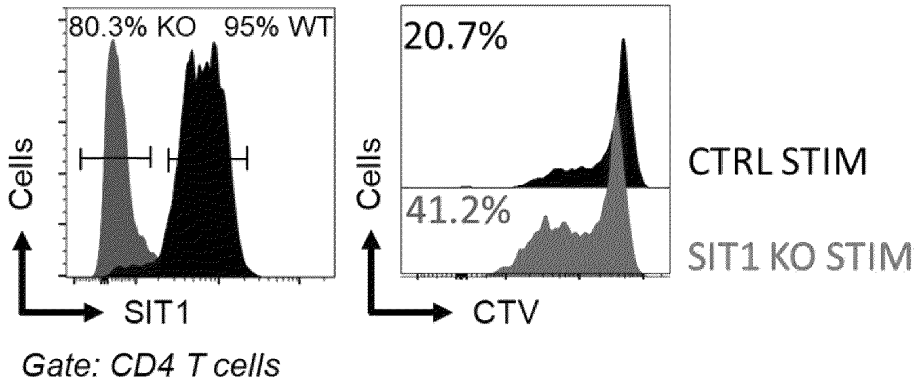

80.3% KO
95% WT
Cells
SIT1
20.7%
41.2%
Cells
CTV
CTRL STIM
SIT1 KO STIM
Gate: CD4 T cells

Figure 30

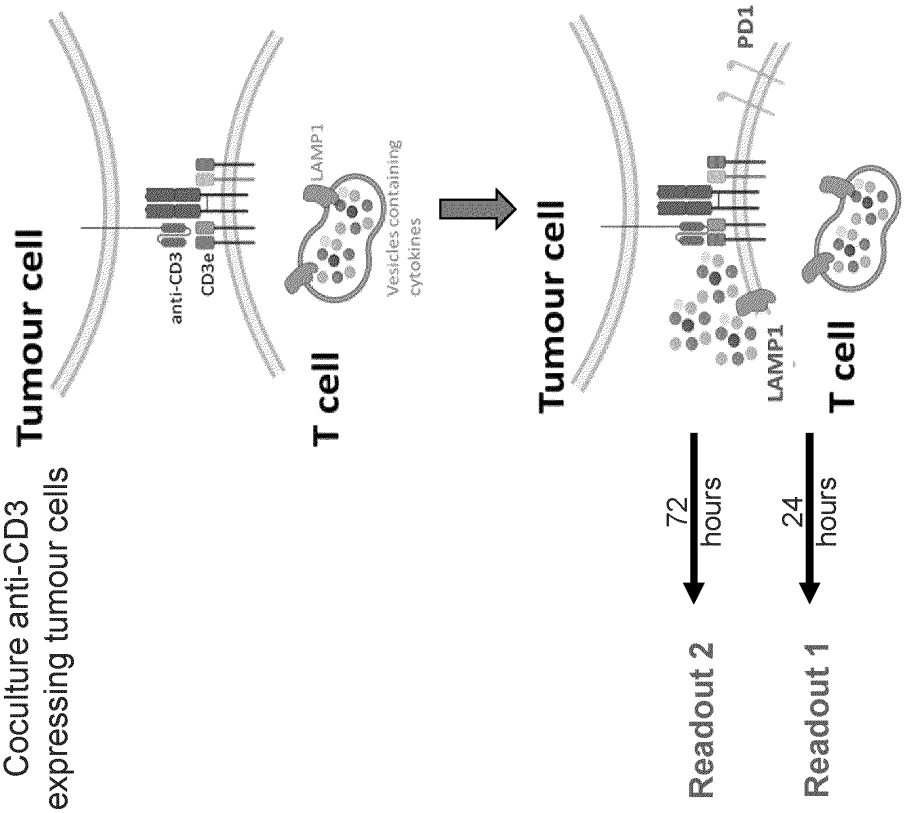

Thaw PBMCs
Activate 3 days
Electroporation of Cas9/crRNA
4 days 50 UI IL2
Coculture anti-CD3 expressing tumour cells
Tumour cell
anti-CD3
CD3e
LAMP1
Vesicles containing cytokines
T cell
Tumour cell
PD1
LAMP1
T cell
72 hours
Readout 2
24 hours
Readout 1

Figure 31A

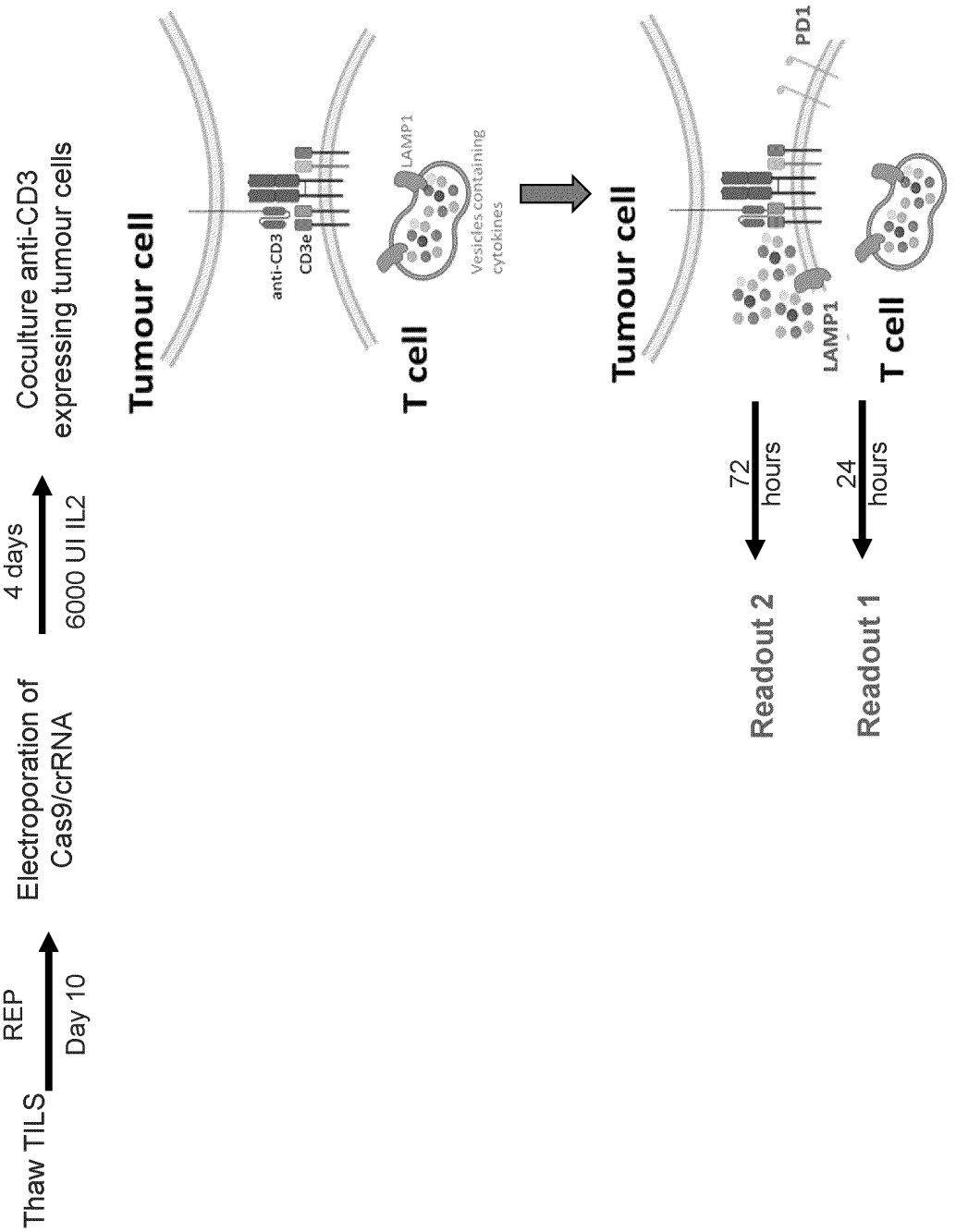

Thaw TILS
REP
Day 10
Electroporation of Cas9/crRNA
4 days
6000 UI IL2
Coculture anti-CD3 expressing tumour cells
Tumour cell
anti-CD3
CD3e
LAMP1
Vesicles containing cytokines
T cell
Tumour cell
PD1
LAMP1
72 hours
Readout 2
24 hours
Readout 1
T cell

Figure 31B

A

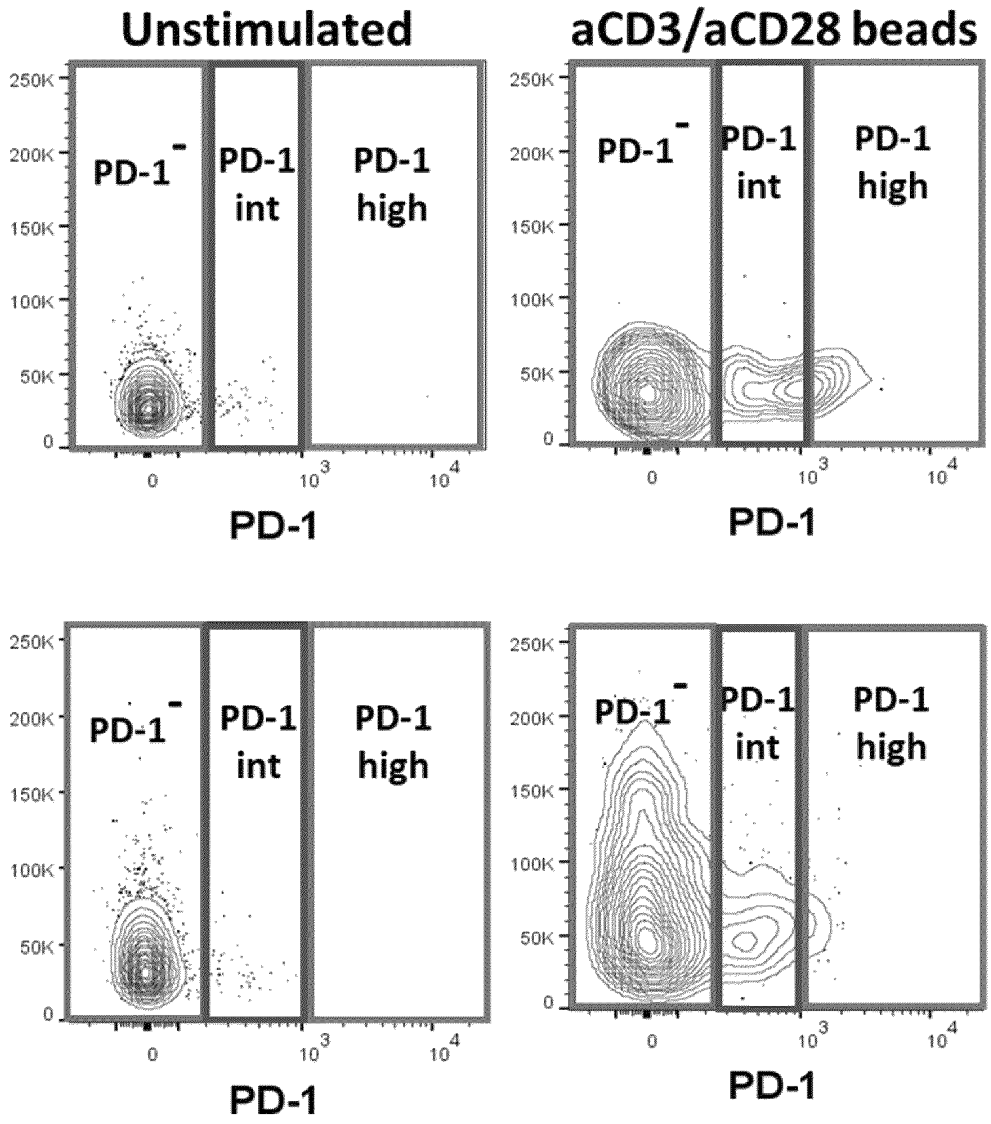

Unstimulated
aCD3/aCD28 beads
PD-1⁻
PD-1 int
PD-1 high
250K
200K
150K
100K
50K
0
0
10³
10⁴
PD-1

Figure 32

B

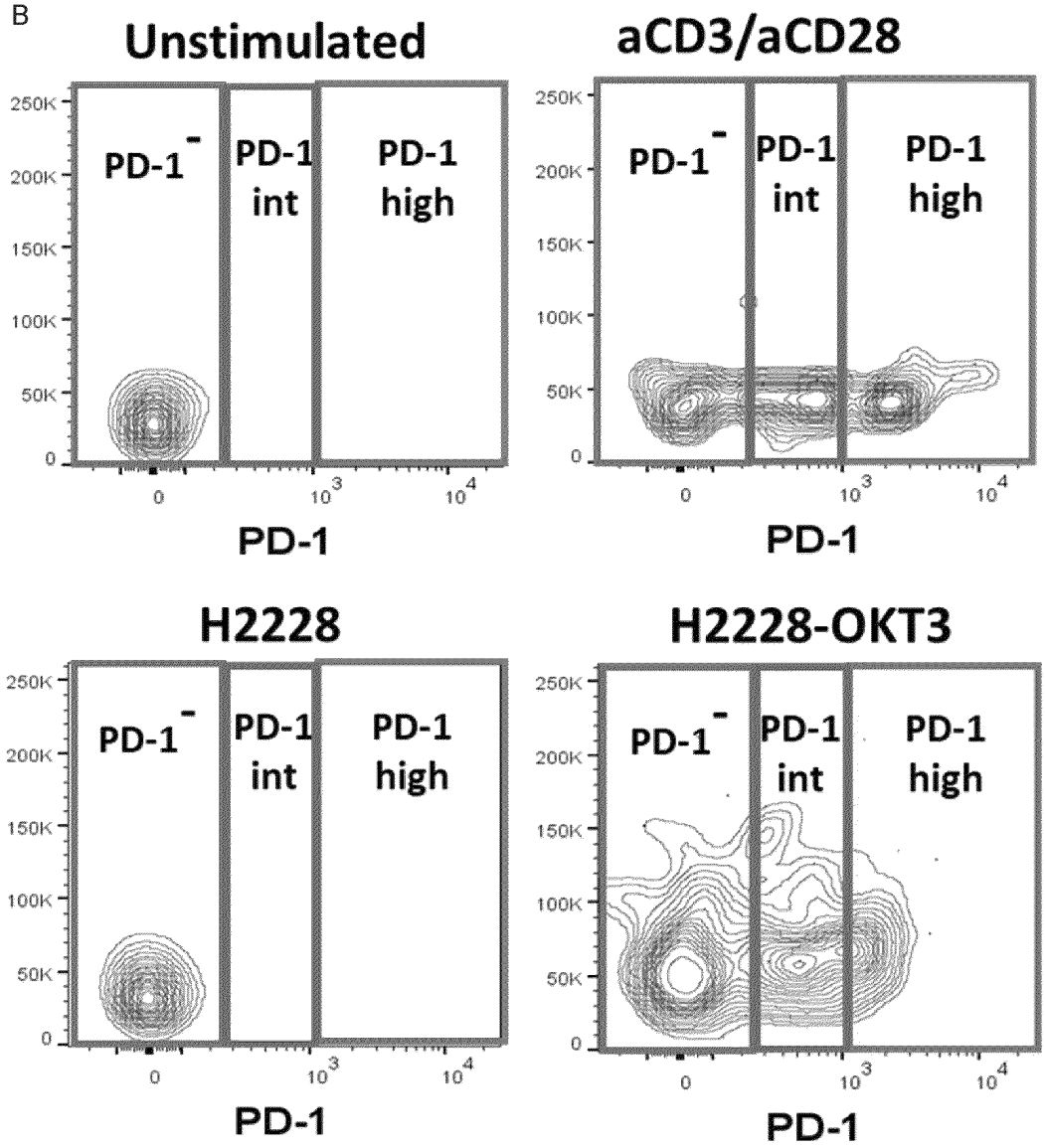


Figure 32 (continued)

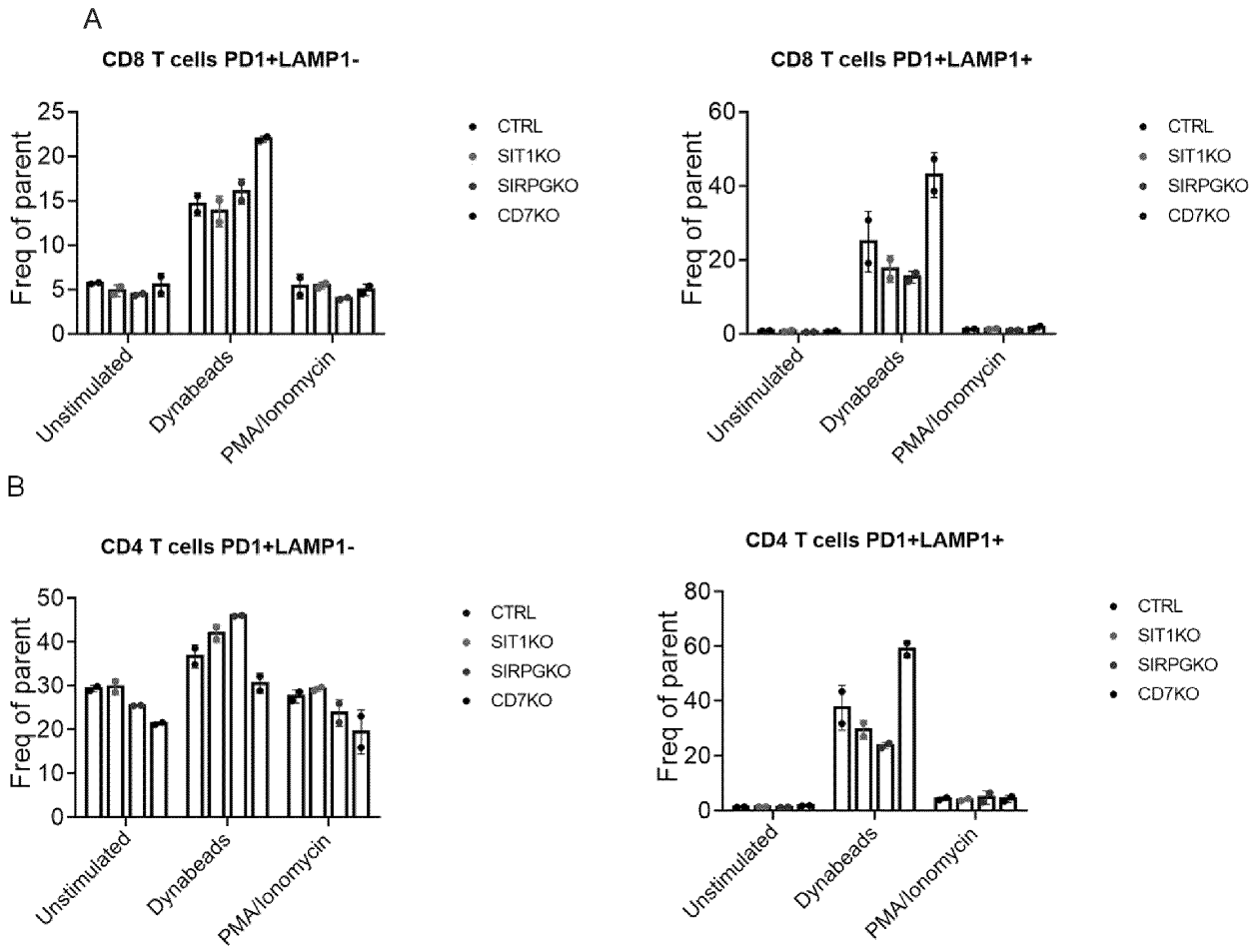
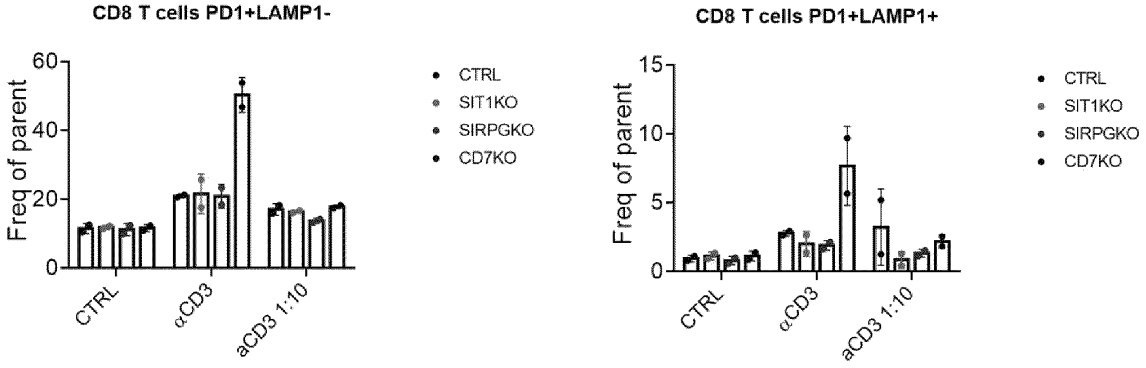
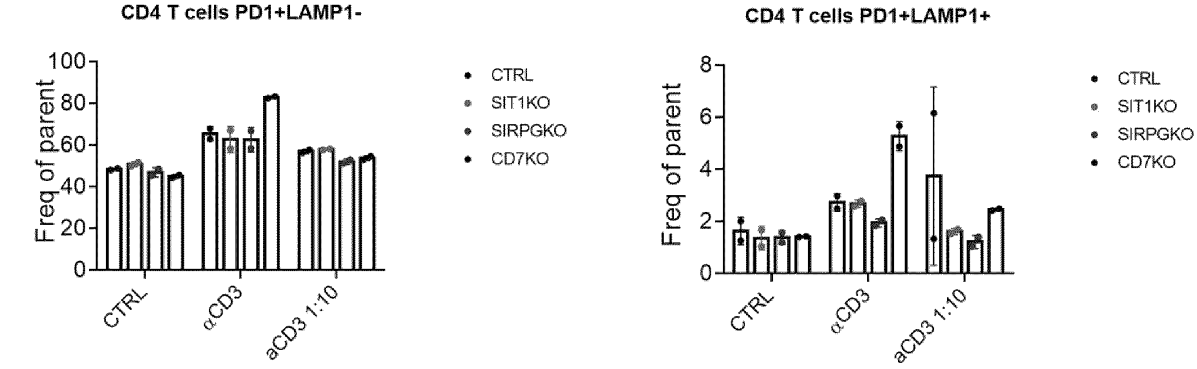

A
CD8 T cells PD1+LAMP1-
Freq of parent
25
20
15
10
5
0
Unstimulated
Dynabeads
PMA/Ionomycin
CTRL
SIT1KO
SIRPGKO
CD7KO
CD8 T cells PD1+LAMP1+
Freq of parent
60
40
20
0
Unstimulated
Dynabeads
PMA/Ionomycin
CTRL
SIT1KO
SIRPGKO
CD7KO
B
CD4 T cells PD1+LAMP1-
Freq of parent
50
40
30
20
10
0
Unstimulated
Dynabeads
PMA/Ionomycin
CTRL
SIT1KO
SIRPGKO
CD7KO
CD4 T cells PD1+LAMP1+
Freq of parent
80
60
40
20
0
Unstimulated
Dynabeads
PMA/Ionomycin
CTRL
SIT1KO
SIRPGKO
CD7KO

Figure 33

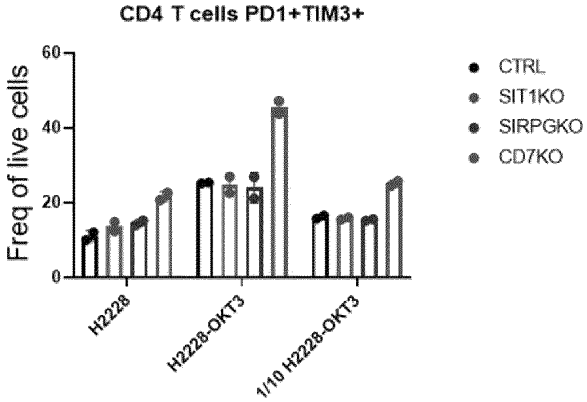
C



D



E



F

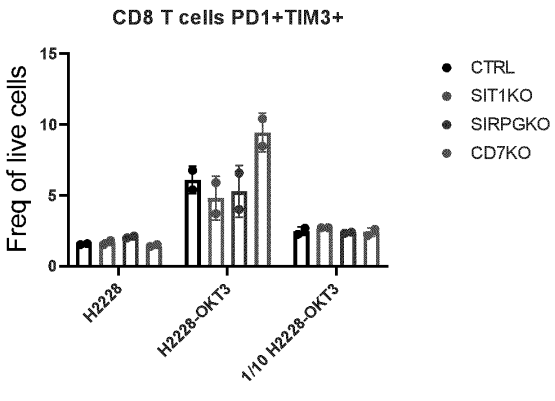


Figure 33 (continued)

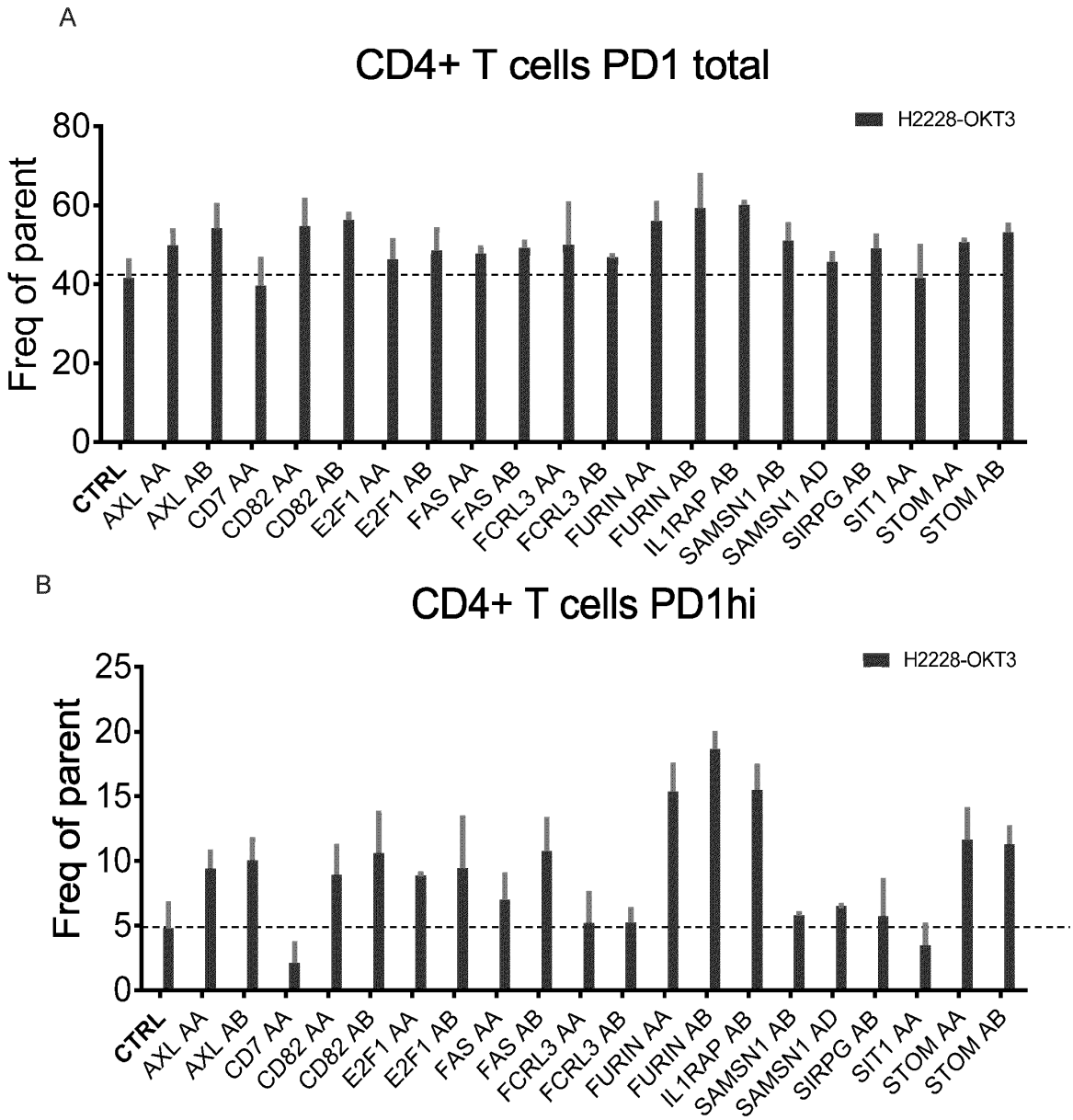
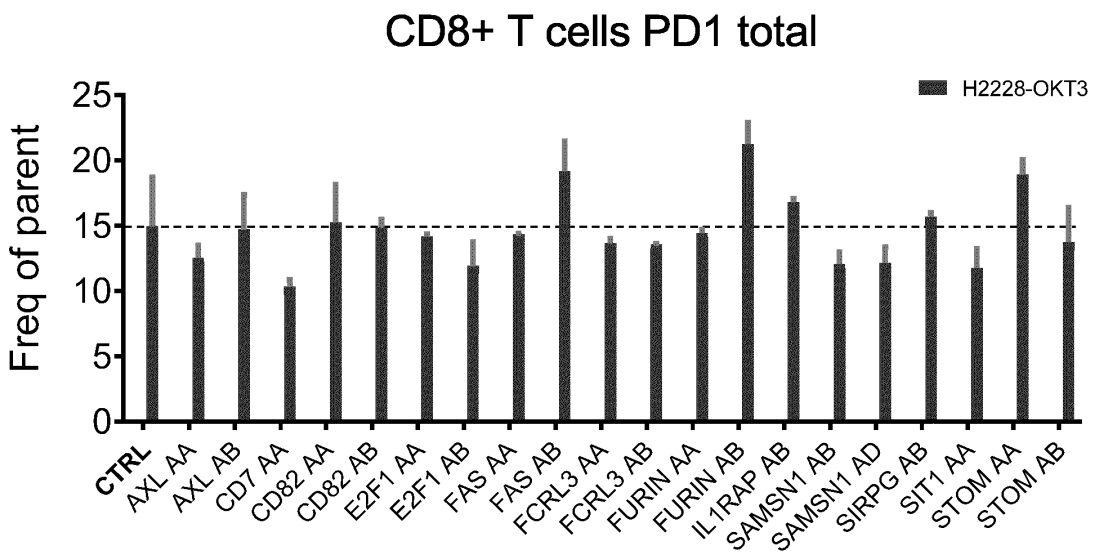

A
CD4+ T cells PD1 total
H2228-OKT3
Freq of parent
80
60
40
20
0
CTRL
AXL AA
AXL AB
CD7 AA
CD82 AA
CD82 AB
E2F1 AA
E2F1 AB
FAS AA
FAS AB
FCRL3 AA
FCRL3 AB
FURIN AA
FURIN AB
IL1RAP AB
SAMSN1 AB
SAMSN1 AD
SIRPG AB
SIT1 AA
STOM AA
STOM AB
B
CD4+ T cells PD1hi
H2228-OKT3
Freq of parent
25
20
15
10
5
0
CTRL
AXL AA
AXL AB
CD7 AA
CD82 AA
CD82 AB
E2F1 AA
E2F1 AB
FAS AA
FAS AB
FCRL3 AA
FCRL3 AB
FURIN AA
FURIN AB
IL1RAP AB
SAMSN1 AB
SAMSN1 AD
SIRPG AB
SIT1 AA
STOM AA
STOM AB

Figure 34

C



D

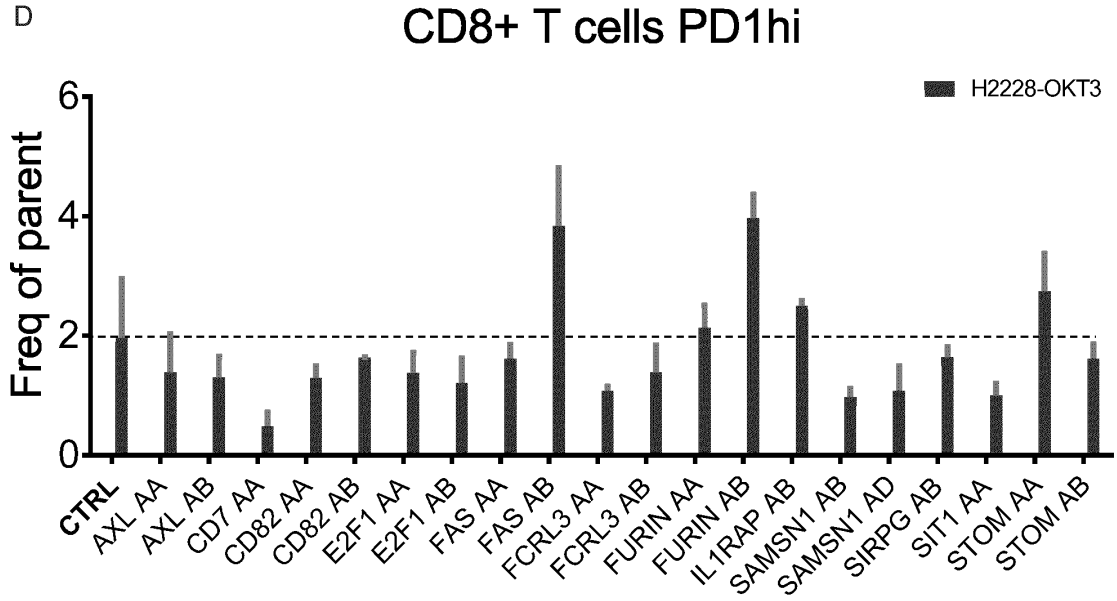


Figure 34 (continued)

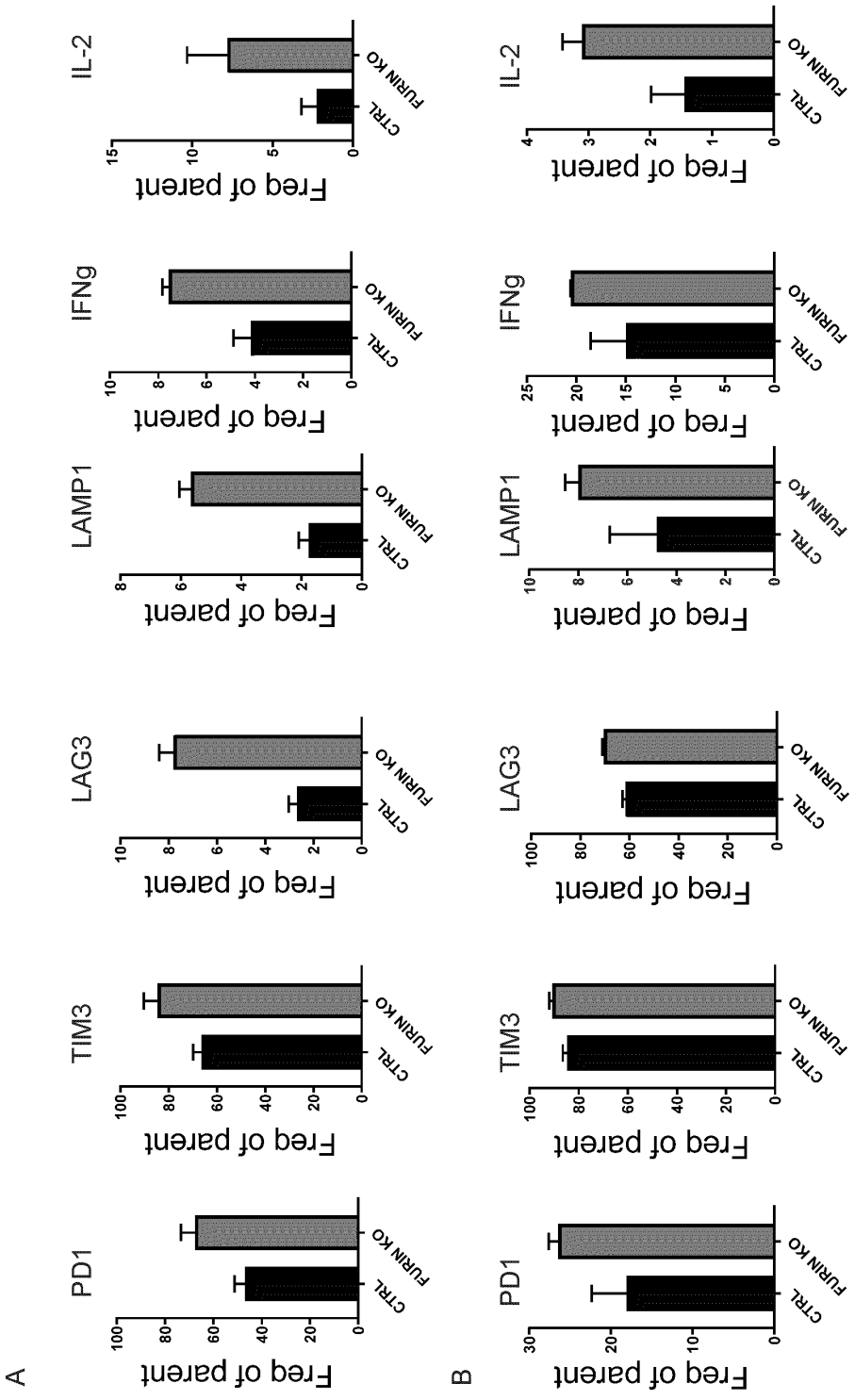


Figure 35

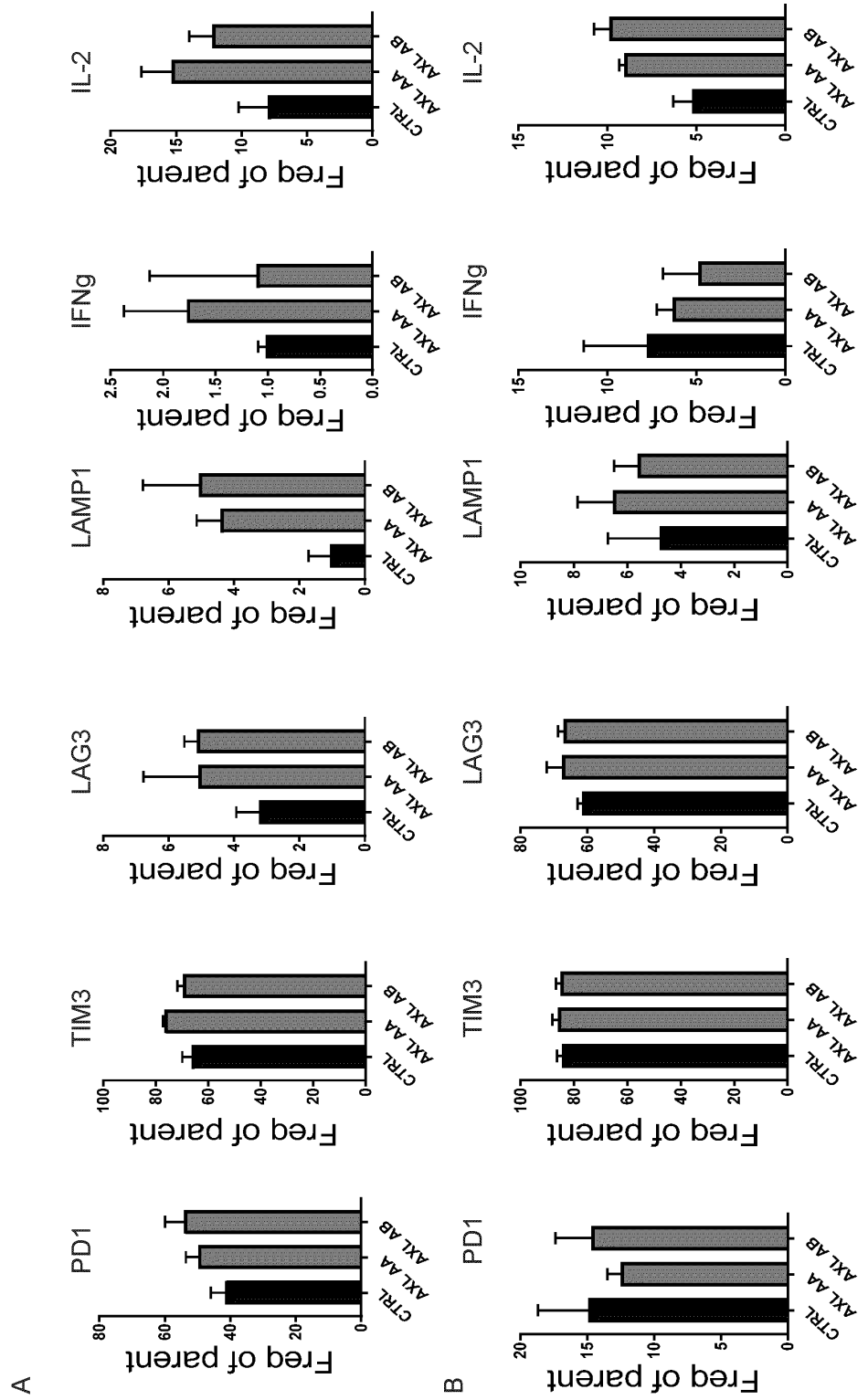

A
B
PD1
TIM3
LAG3
LAMP1
IFNg
IL-2
Freq of parent
CTRL
AXL AA
AXL AB

Figure 36

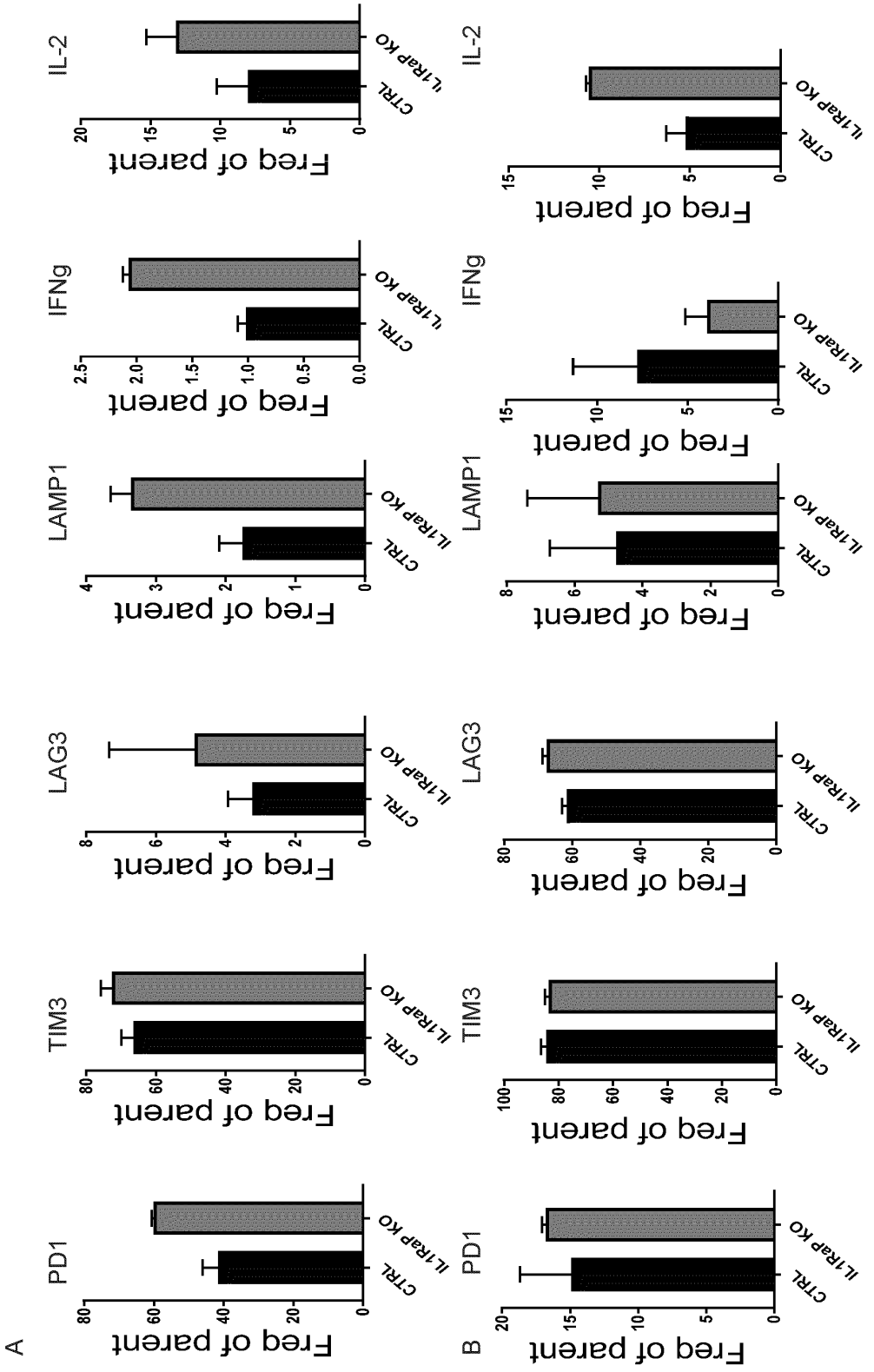


Figure 37

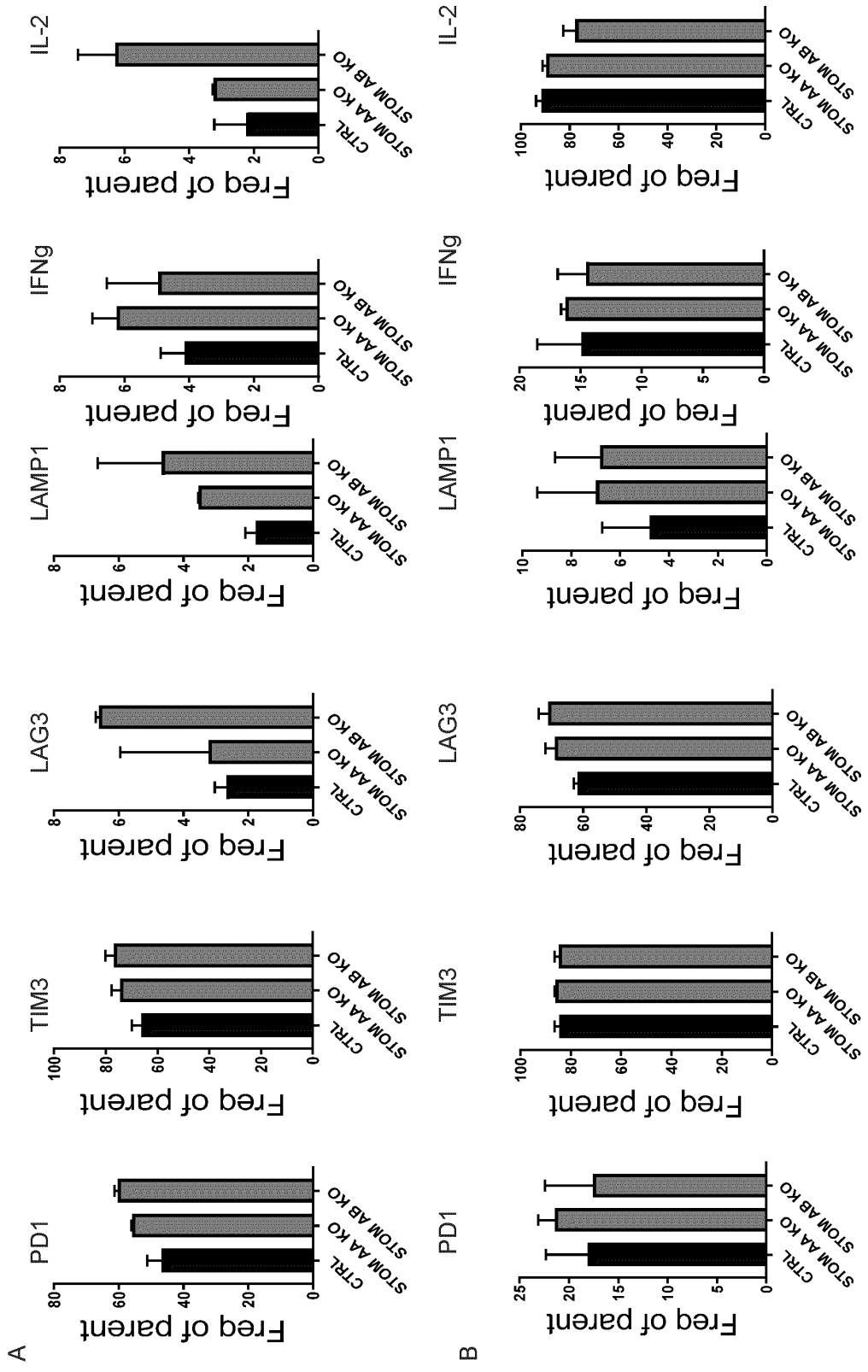

A
B
PD1
TIM3
LAG3
LAMP1
IFNg
IL-2
Freq of parent
CTRL
STOM AA KO
STOM AB KO

Figure 38

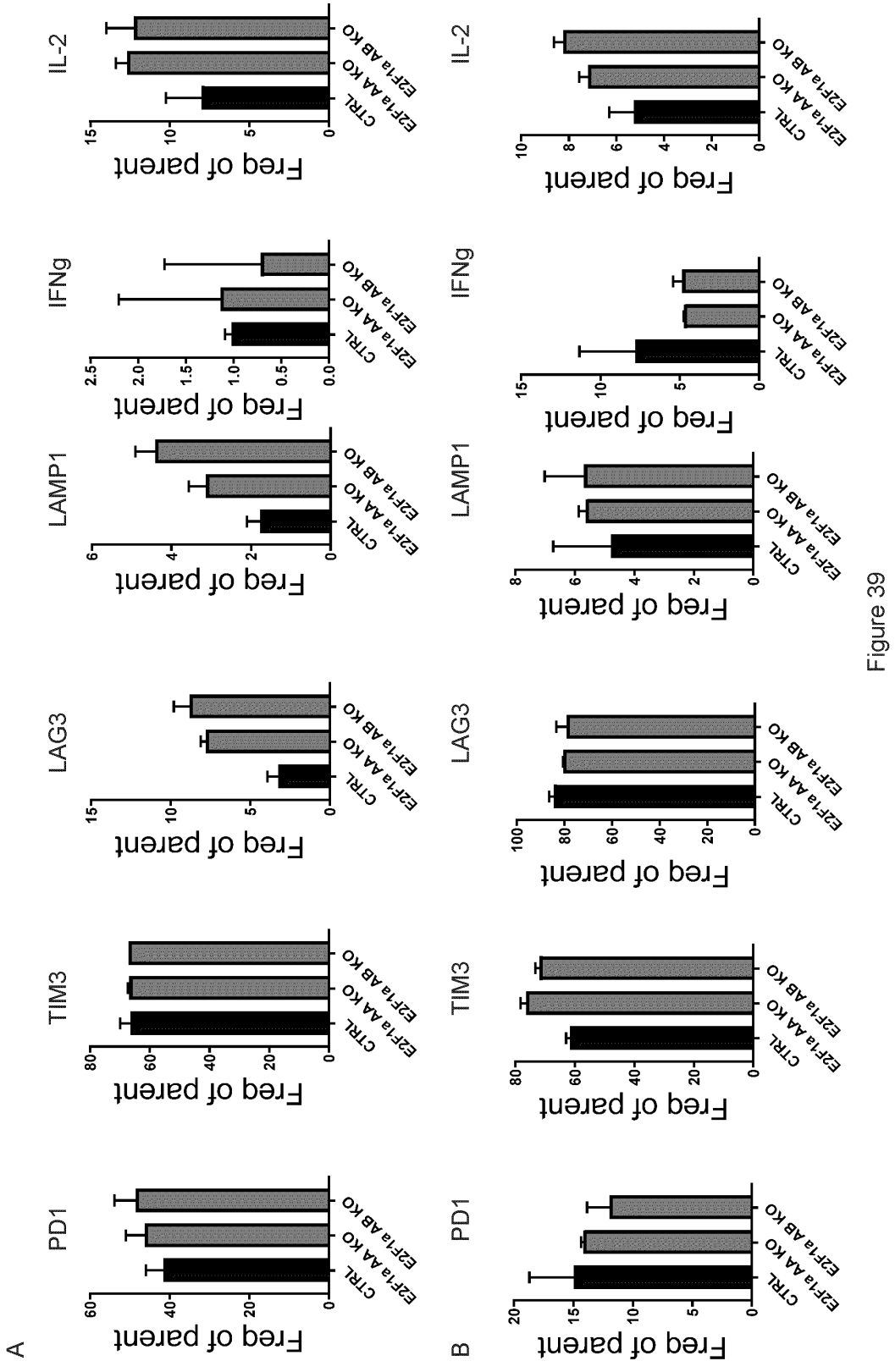

A
B
PD1
TIM3
LAG3
LAMP1
IFNg
IL-2
Freq of parent
CTRL
E2F1a AA KO
E2F1a AB KO

Figure 39

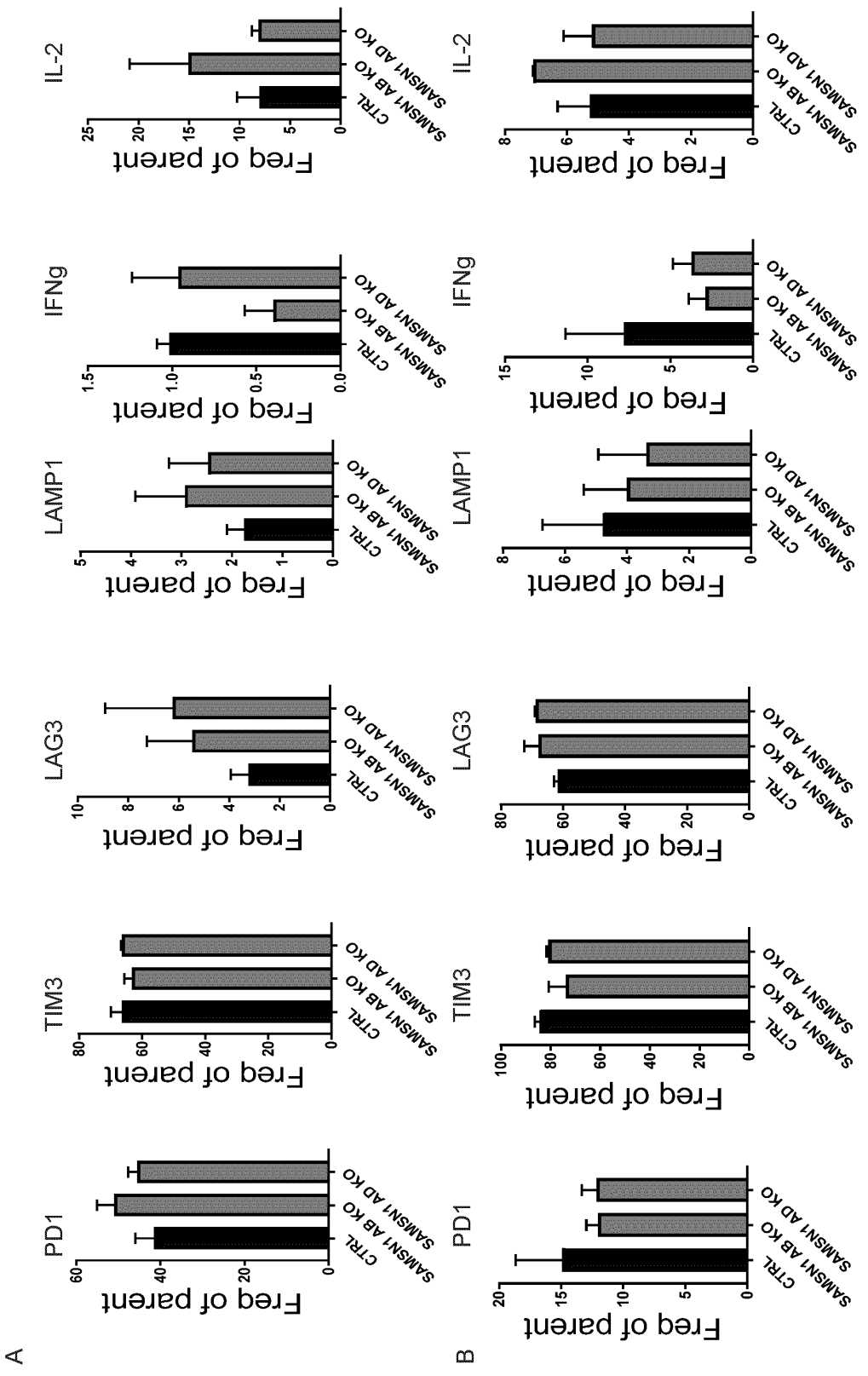

A
B
PD1
TIM3
LAG3
LAMP1
IFNg
IL-2
Freq of parent
CTRL
SAMSN1 AB KO
SAMSN1 AD KO

Figure 40

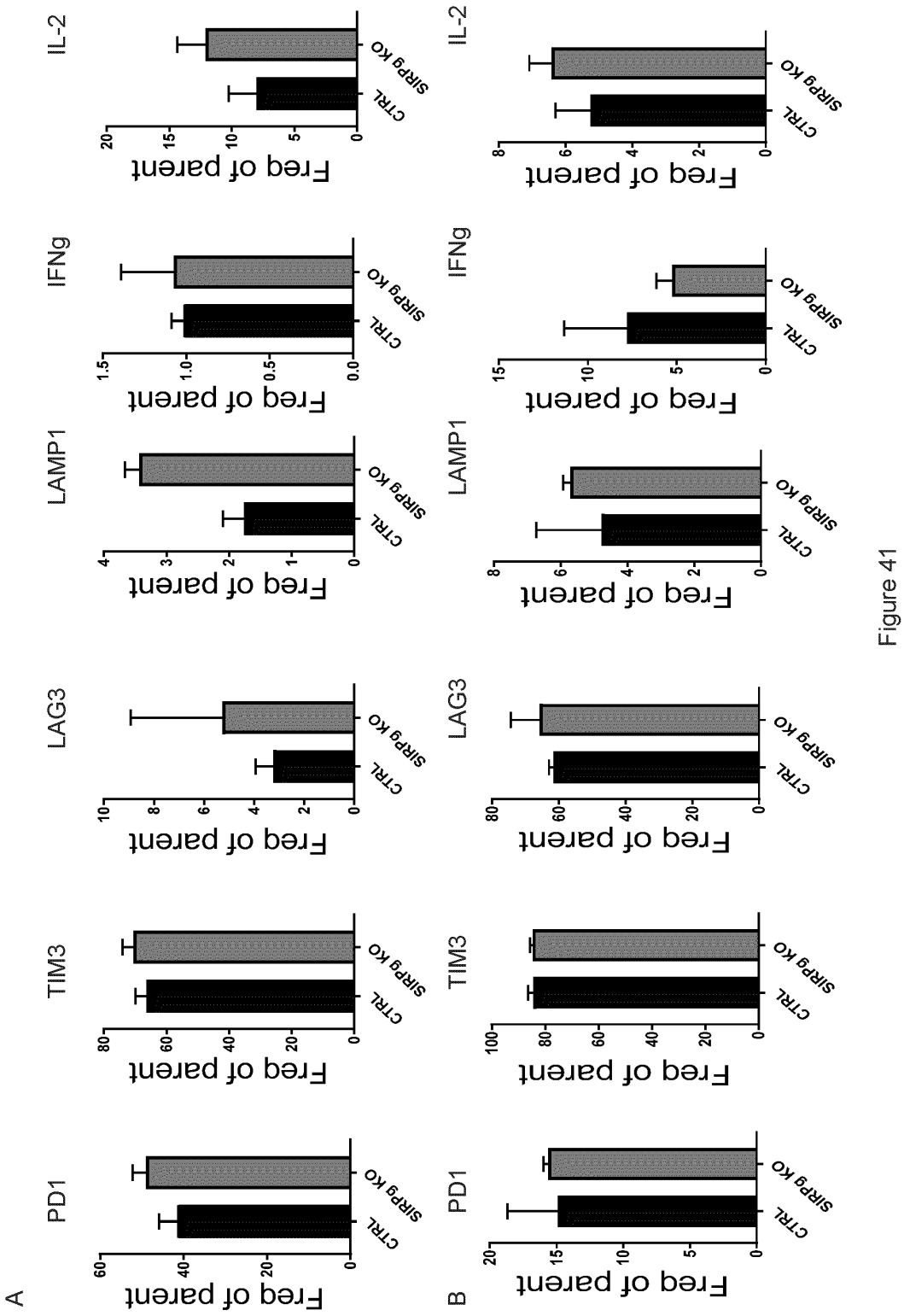


Figure 41

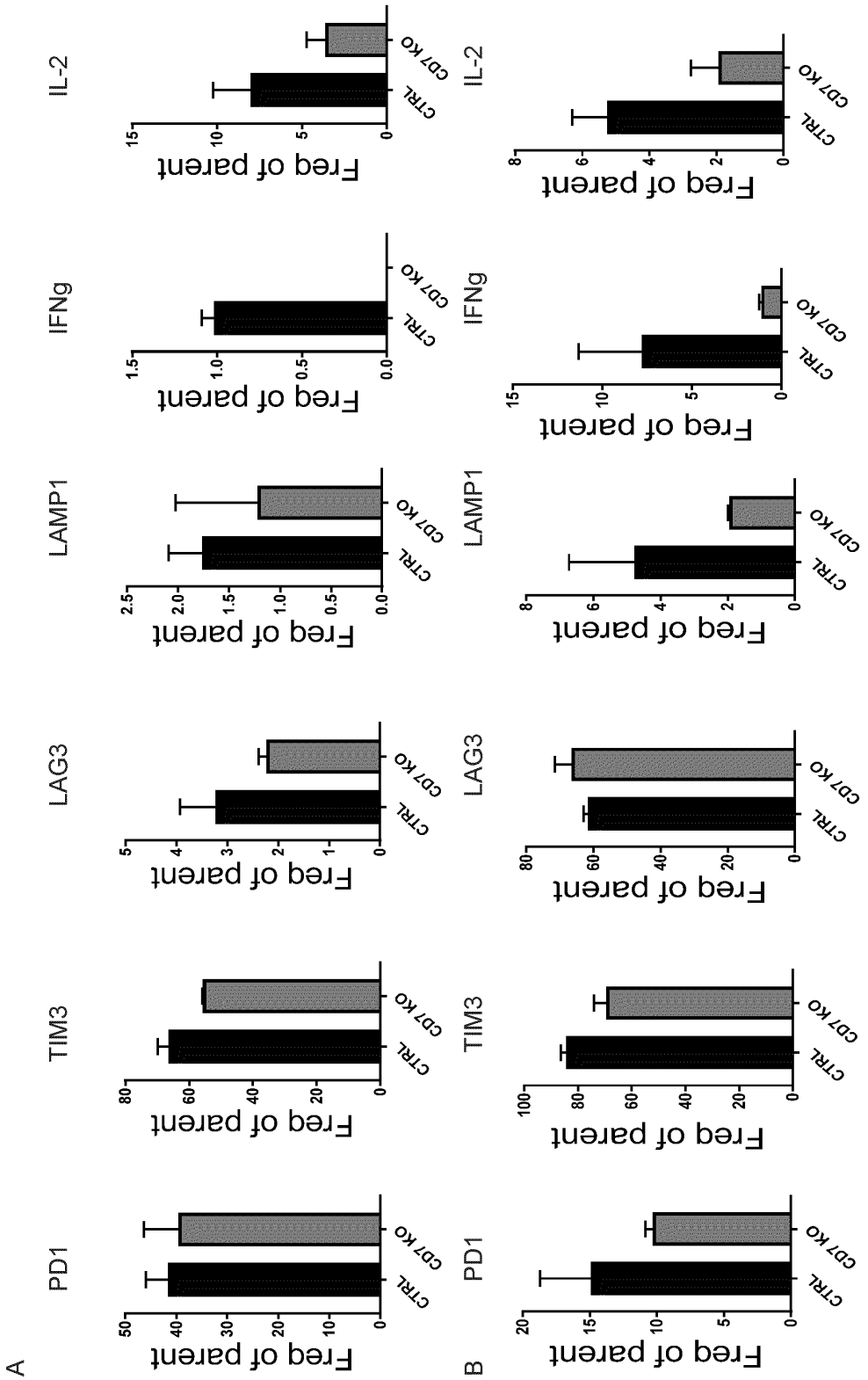


Figure 42

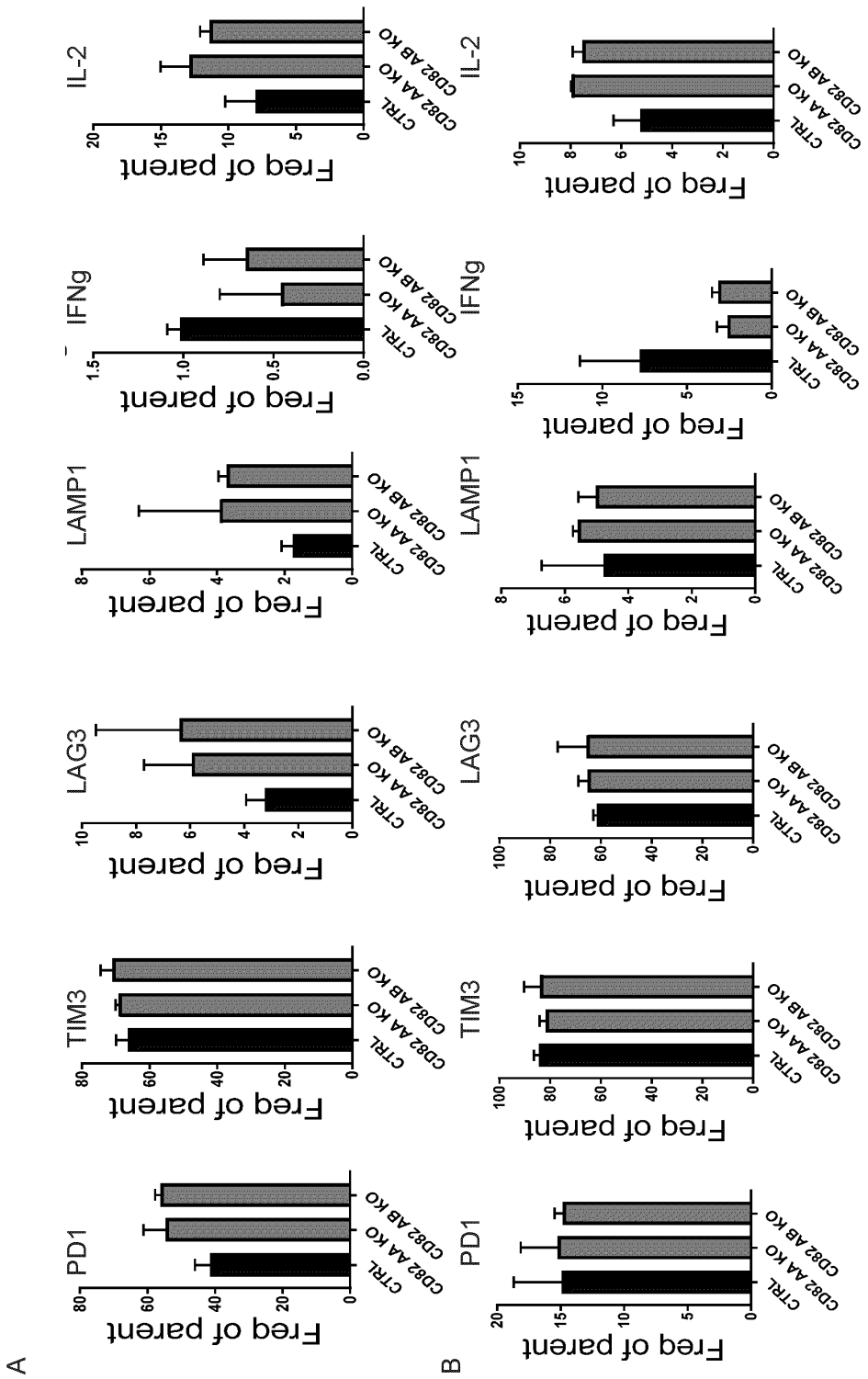

A
B
PD1
TIM3
LAG3
LAMP1
IFNg
IL-2
Freq of parent
CTRL
CD82 AA KO
CD82 AB KO

Figure 43

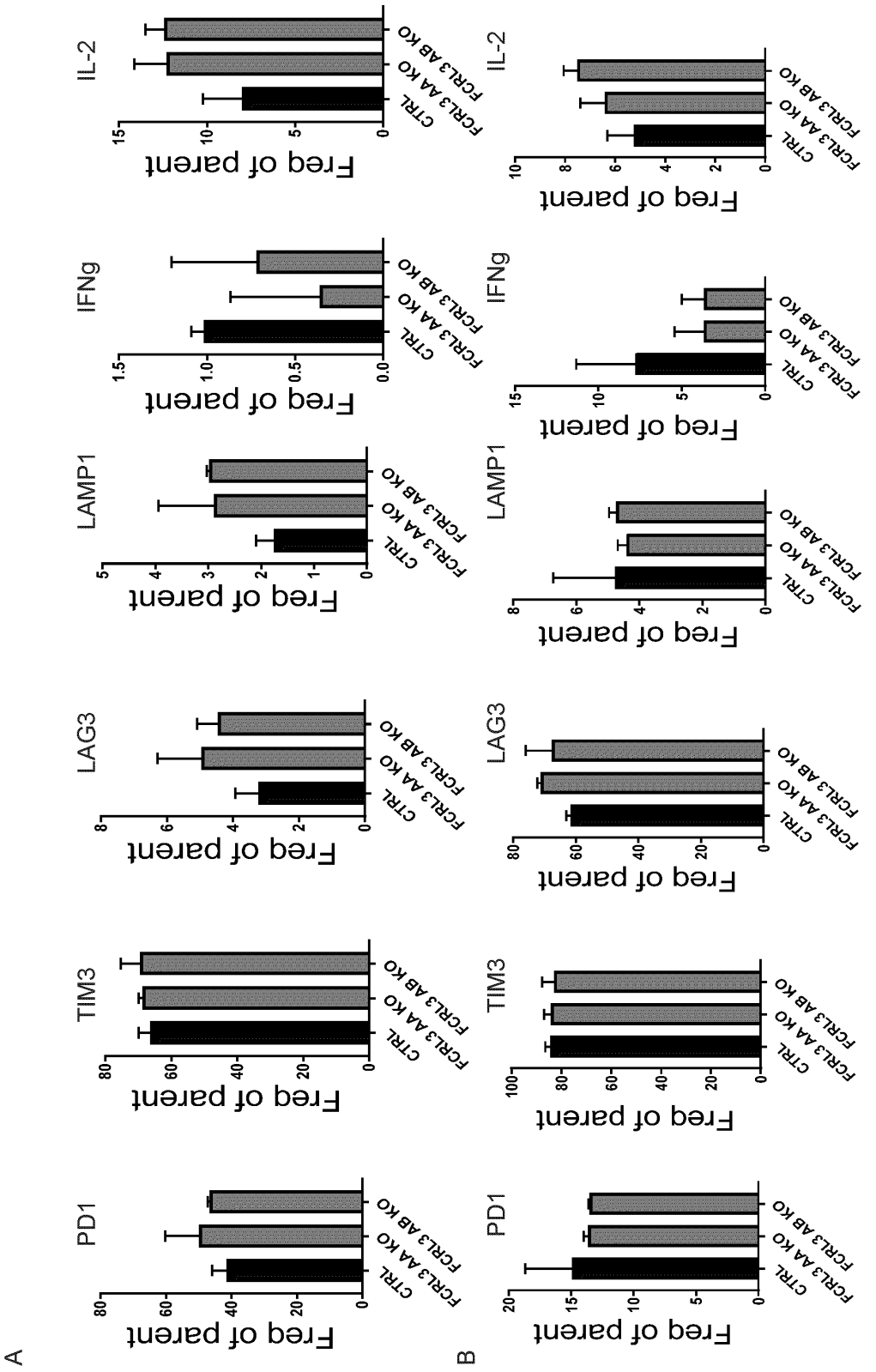

A
B
PD1
TIM3
LAG3
LAMP1
IFNg
IL-2
Freq of parent
CTRL
FCRL3 AA KO
FCRL3 AB KO

Figure 44